THE ALLEGED EXISTENCE OF ADRENALIN (EPINEPHRIN) IN PATHOLOGICAL SERA.*

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Plates 77–80.

In a previous paper,[1] I concluded that no proof had hitherto been given that adrenalin is ever present in detectable amount in normal sera from the general circulation. I have since examined by similar methods a number of sera from clinical cases. In the first paper, the literature on the alleged detection of adrenalin in pathological sera and the so-called estimation of the variations in the adrenalin content of the blood in different diseases was sufficiently covered, and few further references will be given here.

In describing my own results, I shall examine and illustrate by special experiments the difficulties in the methods hitherto employed which, in my opinion, render the search for adrenalin under any conditions in the general circulation quite unpromising and at present devoid of practical importance in clinical work. As will be shown, the methods used do suffice for the detection of adrenalin in serum obtained more or less directly from the adrenal glands when they are excited to activity. Accordingly, the failure of the methods in the case of the general mixed blood is due, not to their inadequacy to detect adrenalin, but to the absence of that substance in the general circulation or to its presence in a concentration below the limit recognizable by the so-called biological tests.

Since these tests, with the objects employed, depend solely on the interpretation of tracings, it is necessary to reproduce a considerable number of these and to refer to them in detail.

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[1] G. N. Stewart, *Jour. Exper. Med.*, 1911, xiv, 377.

TECHNIQUE.

The technique was the same as that described in the previous paper, segments of rabbit small intestine and of rabbit uterus constituting the test objects. The animals were killed by a blow. In a few experiments the rabbit was kept under urethane given by stomach tube. But this seemed to cause irregularity in the beats of the intestinal segments, rendering them somewhat unsatisfactory till after repeated washing. The oxygen stream entered the apparatus by a side tube opening low in a small cylinder. The tube was of capillary bore so as to give small bubbles of oxygen, and at the same time to take up only a little of the liquid in the cylinder.

It is scarcely necessary to mention that the steadiness and sufficiency of the oxygen stream were sedulously controlled. Otherwise grave errors of interpretation of the changes in the curves can very easily occur. For example, in figure 1 an intestine preparation was beating in Ringer. At 6 the oxygen supply was increased and a great increase in the size of the beats occurred, both the maximum contraction and the maximum relaxation being markedly augmented. At 6 in figure 31 precisely the opposite effect at first accompanied the increase in oxygen; viz., a great drop in tone, succeeded by a more lasting and steady rise.

The condition of the preparation at the moment is, without doubt, an important factor in relation to the effect of variations in the oxygen supply. In figure 8, with a uterus preparation which was contracting rather feebly in Ringer, an increase in the oxygen at 6 caused a prompt and great increase both in the individual beats and in tone, which, if it had coincided with the addition of a suspected serum, might easily have been taken for an adrenalin effect.

The liquid in the cylinder was always replaced by running the new liquid into the bottom of the cylinder and allowing the displaced liquid to overflow into the bath. A separate narrow tube piercing the rubber cork which closed the bottom of the cylinder and connected with a small funnel was first employed for this purpose, but it was soon discarded as presenting no advantages, and in the great majority of the experiments the new liquid was added by passing a pipette drawn to a fine point down to the bottom of the cylinder. The pipette was slipped down along the glass and did not interfere at all with the preparation. The pipette was bent at a right angle so that when the point was in the cylinder its other limb, connected with a small rubber tube and glass mouthpiece, was horizontal. The horizontal limb was graduated so that the amount of liquid introduced was accurately known. As a rule, it was just enough to fill the cylinder, and displaced, therefore, all the liquid previously present. Thus the preparation was always completely immersed in liquid and the disturbance caused by alternately emptying and filling the cylinder, during which the preparation alternately sinks to the bottom and is floated up again to the top, being now in contact with gaseous oxygen and now with liquid, was avoided.

The apparatus is conveniently made in different sizes by cutting down glass T-pieces. Half a dozen cylinders of different capacity were constructed but two were used mainly: one, referred to as the larger apparatus, holding without the preparation 4.5 c.c.; the other, referred to as the smaller apparatus, holding without the preparation 2.5 c.c. When the supply of serum was abundant, the former was used; when it was scanty, the latter. When the preparation was in

position, 4 c.c. of serum or other liquid sufficed completely to displace the contents of the larger apparatus, and 2 c.c. to displace the contents of the smaller.

The apparatus used and the weight of the preparation after drying with blotting paper were always noted since the action of a substance such as adrenalin is influenced, not only by its concentration, but also by the relation its total amount bears to the mass of the tissue affected.

What has just been said of adrenalin is true also of serum. The necessity of controlling this factor is well illustrated in figure 2. At 33, while an intestine preparation was contracting rather feebly in Ringer, 1 c.c. of dog serum was introduced into the small apparatus. It displaced about half of the Ringer and was soon mixed up with the rest by the bubbles of oxygen forming a serum diluted with its own volume of Ringer's solution. The size of the beats was much increased with very little increase of tone. At 34, 2 c.c. of the serum was run in, displacing all the diluted serum, and a marked increase of tone occurred.

The preparation was always tested with adrenalin solutions of different concentrations, made up in the suspected serum, or in a control serum, or in Ringer's solution, or in all three. Such comparisons for each preparation are essential.

The adrenalin chlorid solution (1:1,000) of Parke, Davis and Company was employed. As a rule, no bottle was used more than three or four days after it had first been opened. For the more important experiments a fresh bottle was invariably opened. The adrenalin was of course kept in the ice chest. After the application of a given serum or solution the preparation was thoroughly washed with Ringer's solution (introduced by the pipette already mentioned) before a new observation was begun. Unless otherwise stated, the liquid tested was introduced while the preparation was beating in Ringer.

COMPLICATING EFFECT OF SERUM ON THE TESTS.

The difficulty of detecting adrenalin in blood serum is greatly enhanced by the fact that serum itself exerts a marked influence on the biological objects which are probably the most sensitive to adrenalin; namely, the intestine and uterus. Serum causes a great increase of tone in both objects. In the case of the intestine, the adrenalin inhibition must overcome the pressor[2] effect of the serum before it can manifest itself. In the case of the uterus, at least in that of the adult uterus which has been once impregnated, the serum and adrenalin effects are in the same direction, both causing an increase of tone. In this case, therefore, the presence of adrenalin in a given serum can be revealed only by an increase in the effect of the serum. The tone-increasing power of foreign serum (horse)

[2] Although the term "pressor" is usually employed by physiologists in relation to increase in tone of blood-vessels, it will be convenient sometimes to use it here to denote an increase in tone of smooth muscle of the intestine and uterus, and no ambiguity can arise from this.

on the smooth muscle of perfused blood-vessels of cats, recently investigated by Schultz,[3] opens to objection the perfusion test of Trendelenberg. Such test objects are, therefore, very much less sensitive for adrenalin when it is in serum or blood than when it is in simple solutions, like Ringer's. This lack of sensitiveness in serum will continue to be a difficulty until means have been adopted either to eliminate the pressor effect of the serum without at the same time destroying the adrenalin or to eliminate the adrenalin without affecting the pressor effect of the serum. The observation of Schultz[4] that, after boiling, the pressor action of serum on segments of intestine is much diminished, is of interest in this connection.

Each specimen of serum, not only from different animals or different normal human beings or different clinical cases, but from different points in the circulation of one and the same animal, or from the same point at different times, might be expected to differ in its pressor activity on the organs with smooth muscle. As a matter of fact, this has, in a large number of instances, been shown to be the case. It is clear that if an intestine preparation is beating in serum A and the replacement of serum A by serum B causes a diminution in tone, it cannot be concluded that this is due to adrenalin or to an adrenalin-like substance, unless it is known that the pressor effect of B (apart from any adrenalin content it may have) is not less than that of A. If it is less than that of A, a fall of the curve may be expected to occur when B displaces A, even if B is altogether free from adrenalin. In the present as in the previous investigation, this difficulty is eliminated as far as possible by using a second smooth muscle preparation (uterus) on which the normal action of adrenalin is to produce an increase of tone. If the fall of tone supposed to have been caused by B in the intestine preparation when it displaced A is due to the pressor effect of B on the smooth muscle being less than that of A, then serum B ought also to cause a fall of tone in a uterus preparation previously beating in serum A. On the other hand, if the action is due to adrenalin or to a sympatho-mimetic substance, B ought to increase still more the tone of the uterus preparation already increased by A. Probably

[3] W. H. Schultz, *Jour. Pharmacol. and Exper. Therap.*, 1912, iii, 299.
[4] W. H. Schultz, *Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 549.

the same would apply to the smooth muscle of blood vessels, which might be substituted for the uterus in discriminating between changes of tone produced by adrenalin and those produced by differences in the pressor activity of different sera for smooth muscle. The complicating effect of serum on the detection of adrenalin added directly to it was illustrated by special experiments in which solutions of adrenalin of different strengths in fresh calf serum were compared with solutions of equal strength in Ringer's.

In figure 3 it is shown that with the volumes of liquid employed (enough to displace the liquid in the larger apparatus) a solution of adrenalin in serum (1 : 10,000,000) produced a distinct preliminary inhibition before the increase of tone due to the serum (observation 31), although the diminution of tone was much less than in observation 33 in the same figure, where, after washing the preparation with Ringer's solution, the latter was replaced by adrenalin (1 : 10,000,000) in Ringer. With the adrenalin-Ringer solution the period of diminished tone was succeeded by a period of increased tone with some increase in the force of the beats, although the maximum tone reached was far less than with the adrenalin-serum. In figure 4 is shown a much more marked preliminary inhibition of the same intestine preparation by adrenalin (1 : 5,000,000) in the calf serum. In figure 5, where a much weaker solution of adrenalin (1 : 25,000,000) in Ringer was made to replace Ringer (observation 20), the inhibitory effect is unmistakable. On the other hand, at 23 and 27, adrenalin (1 : 25,000,000) in the serum produced little if any preliminary inhibition of the intestine, although the effect of the adrenalin might possibly be recognized and to a certain extent estimated by comparing the amount and especially the duration of the increase in tone in the serum-adrenalin curves with the same changes in the serum curve (observation 25). But the fact that even with the conditions kept as constant as possible the increase of tone was much more transient in 23 than in 27, although in both cases it was distinctly less persistent than in 25, where the serum alone was added, shows how difficult it would be to detect adrenalin in serum with certainty in anything like this dilution, even where the control serum is identical with that containing the adrenalin, a condition of course never realizable in testing serum for clinical purposes.

In figure 6, with the same intestine preparation, adrenalin in serum (1 : 50,000,000, observation 35) produced an effect so little different from that of the serum itself (37) that its detection could not be relied on. The somewhat quicker descent after the maximum increase of tone in observation 35 was too little marked to be used as a criterion. With a solution of adrenalin in Ringer of the strength of 1 : 50,000,000 added to the same preparation after washing with Ringer, the typical adrenalin reaction was perfectly distinct (observation 39, figure 6). In these observations, then, where the adrenalin was actually added to the serum, and where, accordingly, the conditions for faultless control experiments existed, the test would unquestionably have failed for dilutions over 1 : 25,000,000, and probably would have been too uncertain for considerably stronger solutions, and this, too, with a sensitive intestinal preparation.

With a preparation which was less sensitive, although from the same rabbit, and not so long isolated (figure 7), a 1 : 5,000,000 dilution of adrenalin in serum was required (observation 7) to give an unequivocal effect as compared with the serum itself (observation 4). At 11 the adrenalin-serum was again added. In both cases although there was a slight initial inhibition, the main effect was a more prompt relaxation after the preliminary rise due to the serum. The maximum increase of tone, however, was practically as great with the serum containing adrenalin as with the normal serum.

In figures 8 to 12 the same serum and solutions of adrenalin in serum were tested on a rabbit uterus preparation. At 5 (figure 8), Ringer was replaced by adrenalin (1 : 25,000,000) in serum, and at 7 by adrenalin (1 : 25,000,000) in Ringer. In figure 9 at 10 and 12, solutions of adrenalin (1 : 50,000,000) in serum and Ringer were similarly compared; in figure 10, solutions of 1 : 10,000,000; in figure 11, solutions of 1 : 5,000,000. In all of these dilutions the presence of serum notably modified the curve, causing a greater increase of tone and a longer and more complete cessation of contractions at the maximum of the tone increase. It is only with quite strong adrenalin solutions that the curve became essentially the same whether serum was present or not. Thus in figure 12, adrenalin in serum (1 : 50,000, observation 29) produced practically the

same effect as adrenalin in Ringer (1 : 50,000, observation 34); *i. e.*, a marked and durable increase of tone and stoppage of contractions.

DETECTION OF ADRENALIN IN BLOOD FROM THE ADRENALS.

We shall now show that the method in our hands is capable of detecting adrenalin in serum when the blood, either undiluted or not greatly diluted with other blood, is obtained from the adrenal gland under conditions favorable to the liberation of adrenalin. This seems all the more necessary as the supposed proof of the liberation of adrenalin as a normal event rests mainly on indirect experiments.

If the question is to be properly tested it is clear that mechanical interference with the gland or with its blood supply must be avoided,[5] for it is more than probable that in asphyxia of the gland or when it is mechanically manipulated a certain amount of adrenalin may get into the circulation. This was tested as follows:

Experiment of February 13, 1912.—A dog weighing 7.5 kilos was anesthetized with ether. A cannula was inserted into the peripheral end of the right femoral vein and a sample of defibrinated blood obtained from it. The serum separated from this blood by the centrifuge is serum I. Then a cannula was inserted into the peripheral end of the transverse lumbar vein which courses over the left adrenal gland between the gland and the inferior vena cava. Care was taken as far as possible to avoid manipulation of the gland. The blood was allowed to escape from the vein while the cannula was being put in and the flow was never interrupted. Then the lumbar vein was ligated before it reached the gland, so that only the adrenal contribution passed through the cannula. A sample of blood was collected from the cannula, which yielded serum II. A third specimen of blood from which serum III was separated, was then taken at once from the adrenal cannula during vigorous massage of the gland with the fingers. Afterwards the rest of the blood in the animal was drawn from the right carotid and the separated serum labelled serum IV. All samples were at once put on ice and the centrifugalizing was conducted in a cool room. The two adrenals together weighed 1.3 grams. The tests with rabbit intestine and uterus (in the small apparatus) were begun within three hours of etherization.

In figure 13, observations 2, 4, and 9 show the increase of tone in the intestine preparation when Ringer's solution was replaced respectively by serum I, serum II, and serum III. Although the maximum increase produced by serum II is somewhat less than the maximum for the other two sera, this as already pointed out is in itself no evidence of the presence of adrenalin. The effect upon the

[5] Kahn, *Arch. f. d. ges. Physiol.*, 1911, cxl, 209.

same intestine preparation of replacing Ringer by serum III (as shown at 6 in figure 14) was very different. There was practically no increase of tone or only a very slight one, while the beats eventually became much smaller. This distinctly suggests the presence of adrenalin in considerable concentration. Observation 11 (figure 13) shows the effect of replacing Ringer by adrenalin 1 : 25,000,000 in serum IV. The maximum increase of tone was much less than that caused by serum IV itself, but there was no preliminary inhibition. The same was true of adrenalin 1 : 12,500,000 in serum IV (observation 13, figure 13), while adrenalin 1 : 12,500,000 in Ringer (observation 15) caused a distinct inhibitory effect. Adrenalin 1 : 1,250,000 in serum IV produced slight inhibition when it replaced Ringer (figure 15, observation 17), but this inhibition was succeeded by a very fair increase of tone, although the beats became much smaller. When Ringer was replaced by adrenalin 1 : 1,250,000 in Ringer at 19, figure 15, the inhibitory effect was quite pronounced. The tone of the preparation beating in Ringer (at 18) was about the same as before 17. In order to save space in grouping the tracings, the relative position of the curves to the base line was not preserved. The increase of tone produced by the adrenalin-serum was so much greater than that caused by serum III in figure 14 as to suggest strongly that if the absence of the pressor effect in serum III was due to its neutralization by adrenalin, the concentration of adrenalin in serum III must have been considerably greater than 1 : 1,250,000. This estimate was confirmed in figure 16 where the suspected serum was caused to replace a control serum. At 28, serum IV replaced Ringer, and at 29 serum IV was itself replaced by serum III. There was an immediate and considerable drop in the curve. At 36 in figure 16, Ringer was replaced by serum IV, and this at 37 by adrenalin 1 : 12,500,000 in serum IV. The inhibitory effect of the adrenalin-serum was so slight and transient that if the effect at 29 was due to adrenalin in serum III the concentration must certainly have been very much greater than 1 : 12,500,000. At 40, Ringer's solution was replaced by adrenalin 1 : 500,000 in serum IV. The fall in the curve was not quite as abrupt and certainly no more marked than at 29. The preliminary rise was exaggerated by accidental interference with the lever during the introduction of the

adrenalin-serum. We can conclude with considerable probability that serum III contained not less than 1 : 500,000 of adrenalin.

By means of the uterus, confirmation was obtained for the conclusion that serum III contained a considerable amount of adrenalin and that the fall of tone produced by it when it displaced serum IV in contact with the intestine was not due to a much inferior tone-increasing power for smooth muscular structures in general. In other words, when, with a uterus preparation, serum III replaced serum IV the tone was still further increased instead of diminished. Four days afterward, the sera having in the meantime been kept in the ice chest, serum III caused only a slight and transient rise when it replaced serum II (figure 24, at 10). Serum II replaced Ringer at 9 in the same figure and produced a good increase of tone.

We may consider it as proved, then, that with an adrenal gland which was not giving off adrenalin to the blood stream in detectable amount, vigorous massage caused the passage into the venous blood coming from the gland of sufficient adrenalin to give a concentration of not less than 1 : 500,000. In a previous experiment in which the cannula was inserted in the same way, but no particular care was taken to avoid disturbance of the gland, both intestine and uterus tests showed adrenalin in blood collected from the cannula. The avoidance of practically all disturbance of the gland was realized in the following experiment.

Experiment of February 14, 1912.[6]—A dog weighing 10.2 kilos was etherized at 8:55 A. M., and carotid and tracheal cannulæ were inserted. The carotid cannula was connected with a manometer. Electrodes were placed on the right and left great splanchnic nerves just central to the adrenals. Ligatures were placed in position around the inferior vena cava; (a) just above the entrance of the adrenal veins, (b) below the entrance of the renal veins. A ligature was also placed in position around the right renal vein and a cannula was inserted through the left renal vein into the inferior vena cava. The ligatures were left quite loose. At 9:37 A. M. sample I (47 c.c. of blood) was taken from the vena cava through the cannula before any of the ligatures had been tightened. At 9:40 sample II (29 c.c.) was taken from the vena cava after tightening all three ligatures so as to make a closed sac of the cava, except for blood from the adrenals and the lumbar veins crossing the glands. The lumbar veins were not tied in order to avoid interference with the glands, and lest the adrenal blood should fill the pocket and cannula so slowly that there would be danger of clot-

[6] This protocol, as well as those of the experiments of February 16, January 19, and January 22, is extracted from the notes of Dr. J. J. R. Macleod, who was good enough to furnish me with the various specimens of blood which I afterwards tested, a very material service in the investigation.

ting. At 9:45 A.M. sample III (50 c.c.) was collected from the cannula in the cava under the same conditions as sample II, except that both splanchnics were stimulated continuously during collection. Before simultaneous stimulation, the nerves were excited separately to test the effect on the blood pressure. The left was more active than the right, although each produced a decided rise. They were stimulated together for six minutes during which time the blood was collected. The venous pocket filled but slowly. After sample III was collected the nerves were again separately tested with regard to blood pressure and were found active. At 9:53 sample IV, consisting of as much blood as could be obtained from the carotid artery, was collected at the end of the experiment. The sera were separated at once by the centrifuge. Samples I and IV were free from hemoglobin; II and III were slightly and equally tinged. All were kept in the cold, and the tests with intestine and uterus from a pregnant rabbit weighing 1,770 grams were begun four hours after the blood was drawn.

In figure 17 at 20, Ringer, in which an intestine preparation was beating, was replaced by serum IV. The usual increase of tone occurred, and when the tracing had become horizontal serum IV was replaced by serum III at 21. An immediate and sharp drop in the curve took place, indicating the possibility that adrenalin was present. At 22, when the tone had recovered somewhat and the curve was again horizontal, serum III was in turn displaced by serum IV and the curve rose again. After washing, the Ringer was replaced at 24 by serum IV, and this at 25 by serum II. Instead of a drop there was no change, or perhaps a slight rise. At 28 Ringer was replaced by serum IV, and this at 29 by adrenalin 1 : 1,000,000 in serum IV. The same prompt drop occurred as in 21. In proportion to the height of the curve at the time the adrenalin serum was added, the drop was fully as great as with serum III and the subsequent recovery of tone was somewhat slower. If, then, the effect in observation 21 was due to adrenalin, it may be concluded that the concentration of adrenalin in serum III was a little but not much less than 1 : 1,000,000. Of course, when adrenalin in Ringer's solution in this concentration (1 : 1,000,000) was added (at 40), with the preparation already beating in Ringer, the inhibitory effect was much more pronounced. The preparation weighed 0.41 of a gram.

Results with a uterus preparation (weighing 0.65 gm.) from the same rabbit are given in figure 18. At 42 Ringer was replaced by serum IV, and this at 43 by serum III, which caused a further increase of tone with diminution or temporary cessation of the contractions at the maximum of the increase in tone. It may accord-

ingly be inferred that the diminution of tone in the intestine caused by serum III when it replaced serum IV was not due to the inferiority of serum III as compared with serum IV in tone-increasing (pressor) power for smooth muscular structures. It must, then, have been due to some substance in it which increased the tone of the uterus while diminishing that of the intestine; *i. e.*, a substance like adrenalin. At 46 (figure 18) serum IV replaced Ringer, and was itself replaced at 47 by serum II. There was no increase of tone, but possibly some fall. At 50 Ringer was replaced by serum IV, and this at 51 by adrenalin 1 : 1,000,000 in serum IV. A prompt increase of tone ensued. It should be noted that the adrenalin-serum was introduced at the bottom of a contraction and the first portion of the rise, probably to the small nick in the curve, would almost certainly have occurred in any case. At 43 serum III was introduced at the top of a contraction. The increase in tone produced by the adrenalin-serum is, therefore, not very much greater than that caused by serum from the vena cava pocket collected during stimulation of the splanchnics. This strengthens the inference from the observations on the intestine that the adrenalin content of serum III was probably somewhat, but not much, less than 1 : 1,000,000.

Observations 53 and 54 (figure 18) are an almost exact repetition of observations 42 and 43 on the same uterus preparation after it had been well washed with Ringer and show that the rise of tone produced by serum III in the first observation was not accidental.

If adrenalin is really present in a serum it is to be expected that on keeping it in this alkaline medium even at a low temperature it will, after a certain time, disappear. Sera III and IV of February 14 were, therefore, again tested after they had stood in the ice chest for a time. The results on the intestine are shown in figure 19. At 52 Ringer was replaced by serum IV and this at 53 by serum III. At 55 Ringer was replaced by serum IV, and this at 56 by adrenalin 1 : 5,000,000 in serum IV. The tone was slightly increased by this adrenalin solution, but a stronger solution of adrenalin (1 : 500,000) in serum IV, when it was made to replace the weaker solution without washing the preparation, caused a fall (observation 57) much greater and more permanent than that caused by serum III. At 59 Ringer was replaced by serum IV, and this at 60 by adrenalin

1 : 1,000,000 in serum IV. The fall of tone is here also much greater and more lasting than that induced by serum III in observation 53, showing that now the latter contains much less than 1 : 1,000,000 adrenalin, whereas forty-eight hours before, judged by the same criteria, it contained but little less than 1 : 1,000,000. Observation 56 indicates that its content is still much more than 1 : 5,000,000. We can conclude that a great part of the adrenalin, but not the whole, has disappeared from the serum in forty-eight hours.

In passing, it may be noted that the absolute amount of the effect produced by serum III at 53 was little if at all inferior to that produced in figure 17 (observation 21). But in figure 19 the sensitiveness of the preparation to adrenalin was much greater. This shows how futile comparisons on different intestine preparations may be.

That some adrenalin was still present in serum III after three days in the ice chest was indicated by observation 29 (figure 20) on a uterus preparation from an animal probably fully grown but not pregnant. At 28 Ringer was replaced by serum I of February 14; the tone was increased and also the size of the contractions. At 29 this serum was replaced by serum III of February 14. A further increase of tone occurred which was of the same order of magnitude as that caused by a 1 : 5,000,000 solution of adrenalin in an "indifferent" serum (observation 26). At 25 the indifferent serum (serum V of February 16, see protocol, page 560) replaced the Ringer in which the preparation was initially beating. As far as can be judged from an experiment like this on the uterus preparation alone, we can perhaps conclude, knowing that we are really dealing with a serum which originally contained adrenalin, that after three days the adrenalin content of serum III has been reduced from nearly 1 : 1,000,000, to not more than 1 : 5,000,000 at most. From observations on the intestine it may be recalled that after the serum had been kept forty-eight hours it was inferred that it still contained much more than 1 : 5,000,000, although much less than 1 : 1,000,000.

So far, then, we have shown that during stimulation of both splanchnics adrenalin is given off to the blood in sufficient amount to cause a concentration of something like 1 : 1,000,000 in the blood obtained from the vena cava pocket. But of course not all this blood was from the adrenal. The adrenal contribution was probably

diluted at least two or three times. The two adrenals together weighed 1.71 grams. In six minutes 50 c.c. of blood was collected from the pocket. If we suppose that the flow through the adrenals is as great as that attributed to the kidney,[7] 150 c.c. per 100 grams per minute;[8] and that stimulation of the splanchnics does not diminish it, this would only give 2.5 c.c. per minute for the adrenal contribution, or 15 c.c. for the six minutes. The concentration of adrenalin in the blood actually issuing from the glands in this experiment during stimulation of the splanchnics was, therefore, probably not less than 1:300,000. Even supposing that the concentration was two or three times greater, about 1:100,000, which must certainly be considered the extreme limit during what is in all probability a state of high functional activity of the glands, it may well be asked what chance there is of detecting adrenalin in the general blood stream in which the feeble trickle from the adrenal veins is diluted to an extreme degree. In a dog weighing 10 kilos probably not less than 2 kilos of blood passes through the heart in a minute,[9] and at least a third of this along the inferior cava from the portion of the body behind the orifices of the adrenal veins. In six minutes this would amount to 4 kilos of "indifferent" blood, into which would be poured during activity of the glands not more than 0.1 mg. of adrenalin giving, for the inferior cava blood, after receiving the adrenal contribution, an excess in adrenalin concentration of not over 1:40,000,000.

To determine whether during stimulation of the splanchnics adrenalin could be detected in blood removed from the inferior cava anterior to the adrenal veins, the following experiment was done.

Experiment of February 16, 1912.—A dog weighing 15 kilos was etherized at 9:55 A. M. Cannulæ were inserted into the trachea and carotid and a cannula into the right femoral artery. The abdomen having been opened, a catheter was passed through the central end of the right femoral vein and the end guided by the finger to a point just anterior to the entrance of the adrenal veins. The point of the catheter could also be seen through the walls of the veins. The catheter was as large as the femoral vein would admit. At 10:48, 45 c.c. of blood

[7] Burton-Opitz, R., and Lucas, D. R., *Arch f. d. ges. Physiol.*, 1908, cxxiii, 553; Burton-Opitz, R., *idem*, 1909, cxxix, 208.

[8] K. O. Neumann (*Jour. Physiol.*, 1912, xliii, p. xxxi) gives a somewhat greater mean flow for the adrenals (2 to 3 c.c. per gm. per minute) in cats and rabbits.

[9] G. N. Stewart, *Jour. Physiol.*, 1897–98, xxii, 159.

(sample I) were collected from the right femoral artery. At 10:50 A. M., 40 c.c. of blood (sample II) were obtained from the catheter in the vena cava. Then during nine minutes from 10:51 to 11:00 A. M., 55 c.c. of blood (sample III) were collected from the catheter during stimulation of both splanchnics. In a preliminary test during which the carotid cannula was connected with a manometer and a blood pressure tracing recorded, it was shown that stimulation of each splanchnic caused a good rise in blood pressure. As the blood had partly clotted in the catheter the flow for sample III was slow. Stimulation was therefore discontinued, the catheter removed, washed free of clot, and reinserted. Then from 11:05 to 11:07½ A. M., 90 c.c. of blood (sample IV) were obtained from the catheter during stimulation of both splanchnics. Stimulation was then discontinued and from 11:11 to 11:14 A. M., 75 c.c. of blood (sample V) were drawn from the catheter. Finally, at 11:15 A. M., 220 c.c. of blood (sample VI) were obtained from the right femoral artery. All the samples were defibrinated and at once centrifuged in the cold. Sera I and VI were free from hemoglobin, serum II contained a slight trace, serum III more than II, although not deeply tinged. Sera IV and V were the same as II in this respect. The tests were begun less than four hours after the blood was obtained.

In figure 21 the Ringer in which an intestine preparation was beating was replaced at 65 by serum I, and this at 66 by serum III. For six or seven beats there was no change of tone or a slight increase, which was succeeded by a gradual fall. At 68 Ringer was replaced by serum I again, with the object of replacing this by serum III when serum I had been allowed to produce its effect on the preparation for a much longer time. At 69 some more of serum I was run into the cylinder as the oxygen stream had displaced some of the serum. A moderate rise of the curve took place bringing it up to the maximum first reached. At 70 serum I was replaced by serum III and a tardy fall ensued just as at 66. This effect is quite different from the adrenalin effect seen in figures 16 and 17. That it was not really due to adrenalin is indicated by observations 75 and 76 (figure 21). At 75 Ringer was replaced by serum II, and this at 76 by serum III, which produced not a fall but a further decided rise. Observations 72 and 73 are exact repetitions of 75 and 76, except that the serum added at 73 was serum IV of February 16. When tested against another presumably indifferent serum (*i. e.*, a serum free from detectable adrenalin), viz., serum VI of February 16, the two specimens of serum (III and IV) collected during splanchnic stimulation gave the same result, a further increase of tone of the intestine preparation (observations 49 and 50, and 81 and 82, figure 22). Earlier in the experiment it was shown with another intestinal preparation that when serum III or serum IV replaced serum I there was no diminution of tone. The fall in the curve succeeding the introduction of serum III at 66 and 70 therefore indicates not that this serum contained adrenalin, in detectable amount, but that its independent pressor effect on this preparation was less than that of serum I.

Figure 22 shows how marked and abrupt the changes in tone of an intestinal preparation may be when different sera, none of which can be shown to contain adrenalin, are caused to replace each other.

At 78 Ringer was replaced by serum VI, and this at 79 by serum V. The pressor effect of serum V was so much greater than that of serum VI that the

curve was practically doubled in height. Had these two sera been added in the reverse order, serum VI must have produced a drop of the tone of the preparation already beating in serum V which might have stimulated an adrenalin effect. At 49 (figure 22) Ringer was replaced by serum VI, and this at 50 by serum IV. At 81 Ringer was replaced by serum VI, and this at 82 by serum III. In both cases the serum collected from the inferior vena cava during stimulation of the splanchnics produced a considerable additional increase of tone, although much less than that caused when VI was replaced at 79 by V.

The uterus likewise yielded no clear evidence of adrenalin in the blood of the inferior vena cava during stimulation of the splanchnics.

In figure 23 at 84 Ringer was replaced by serum II, which produced an increase in the tone and also in the rate of the contractions. At 85 serum II was replaced by serum III, which caused no further increase of tone, but, if anything, some inhibition, while adrenalin 1:1,000,000 in serum VI when it replaced serum VI at 93 occasioned a good rise. Serum VI, replacing Ringer at 92, caused the usual great increase of tone. In figure 24 at 2 Ringer was replaced by serum VI, and this at 3 by serum III, which certainly produced no further increase of tone. On the other hand, adrenalin (1:1,000,000 in serum VI) caused on replacing serum VI at 16 a very marked rise in the curve, the writing point going above the drum. The preparation was a fresh segment of a horn of the uterus used for observations 2 and 3 (figure 24), and was beating in Ringer when serum VI was added at 15. The replacement of serum V by serum III at 23, likewise caused no further change in the uterus curve which could be interpreted as indicating that serum III contained adrenalin. The replacement of Ringer at 22 by serum V increased the tone after an initial diminution.

Lest the increase of tone in the uterus occasioned by undiluted serum should have rendered it impossible for any clear further increase to occur when one of the control sera was replaced by a suspected serum, the observations illustrated in figures 23 and 24 were repeated with sera diluted with an equal volume of Ringer. In figure 25 an example of the results is given. Qualitatively they differ in no way from the others. At 5 Ringer was replaced by diluted serum VI, and this at 6 by diluted serum III, which in turn was replaced at 7 by diluted serum VI. Another experiment in which blood was collected in the same way from the cava (only the left splanchnic being stimulated) yielded similar negative results.

We conclude from all these observations that although the methods used clearly demonstrate the presence of a considerable concentration of adrenalin in blood coming from the veins of the adrenal glands during electrical stimulation of the splanchnics, they yield no unequivocal evidence of its presence in blood obtained from the inferior vena cava of dogs just anterior to the entrance of the adrenal veins during stimulation of these nerves. The cause of the negative result in this case is without doubt the high degree of dilution in the inferior cava of the blood coming from the adrenals.

Cannon's[10] positive result in cats under the influence of nicotine or emotion[11] might be explained by assuming (*a*) that these stimuli liberate more adrenalin than electrical stimulation of the splanchnic, or (*b*) that in a small animal a catheter produces a more effective block in the inferior cava, and thus allows the adrenal blood to be collected with less dilution.

Elliott's[12] failure to demonstrate exhaustion of adrenalin from the gland itself by electrical stimulation of the splanchnic may be due either to too short a period of stimulation or to too rapid exhaustion of the nervous path. The adrenalin discharged during six minutes of splanchnic stimulation in our experiment of February 14 was not more than 0.05 mg. for each gland. The glands weighed three to four times the average for Elliott's cats. With a six minute splanchnic stimulation probably little more than 0.01 mg. would disappear from a cat's adrenal. Elliott's adrenalin assay was accurate only to 0.01 mg. A cerebral puncture caused the disappearance of 0.3 mg. of adrenalin from one gland in four hours. Suppose that in four hours 150 kilos of blood passed through the cat's heart. If the liberation of adrenalin proceeded uniformly throughout the four hours and new formation is excluded, the liberated adrenalin could only maintain in the cava blood anterior to the adrenal veins an excess in concentration of 1 : 160,000,000. Even if the discharge of adrenalin by one gland is not augmented when the splanchnic supplying its fellow is cut, the liberation of 0.3 mg. from each gland in four hours would only bring up the excess to 1 : 80,000,000.

In two experiments venous blood from the adrenal gland was collected without admixture during stimulation of the corresponding splanchnic. For the reasons already given these experiments were not considered completely satisfactory. In both evidence of adrenalin was found in the blood from the adrenal.

Experiment of January 22, 1912.—Under ether anesthesia sample I was collected at the beginning of the experiment from a femoral vein of a dog weighing 10 kilos; sample II from the left adrenal vein before stimulation of the splanchnic; sample III from the left adrenal vein during stimulation of the left splanchnic. Sample IV, a mixture of blood from the femoral vein and carotid artery, was taken at the end of the experiment and after stimulation of the splanchnic was over; sample V consisted of a portion of the blood drawn from the carotid artery at the end of the experiment; and sample VI constituted the rest of the specimen of which sample V was a portion. Sample VI was evacuated as thoroughly as possible with the gas pump. As in the other experiments of this nature the activity of the splanchnic on the blood pressure was established by a preliminary test. In figures 26, 27, and 28 are seen specimens of the reaction of the intestine after the sera had stood for twenty-four hours in the ice chest. The smaller apparatus was used. The preparation weighed 0.39 gm.

[10] Cannon, W. B., Aub, J. C., and Binger, C. A. L., *Jour. Pharmacol. and Exper. Therap.*, 1911–12, iii, 379.

[11] Cannon, W. B., and de la Paz, D., *Am. Jour. Physiol.*, 1911, xxviii, 64.

[12] Elliott, T. R., *Jour. Physiol.*, 1912, xliii, p. xxxii.

In figure 26 Ringer was replaced at 2 by serum VI and at 4 by serum V. Incidentally it is seen that the evacuation of the carbon dioxid did not affect the tone-increasing power of the serum. At 6 and at 8 Ringer was replaced by serum III, the preparation having been washed during the interval. The two curves are as nearly identical as can be expected, even under fairly constant external conditions, with an object like a segment of intestine. A distinct initial inhibition was, therefore, caused by serum III, and this was absent with V or VI. The inhibition was followed by increase of tone much less than that produced by the supposedly indifferent sera V or VI.

A point of considerable interest is that the inhibition caused by serum III was distinctly more marked than when that serum was tested in the same way three hours after the withdrawal of the dog's blood, necessarily, of course, on an intestine preparation of another rabbit. This suggests that the pressor effect of a serum may diminish more rapidly on keeping than the adrenalin effect.

In figures 27 and 28 the effect of serum II on the intestine preparation used for figure 26 is shown. At 10 the Ringer was replaced by serum II, and after washing the preparation, the experiment was repeated exactly at 12. There was again in both observations evidence of inhibition preceding the moderate increase of tone. At 14 serum I replaced Ringer and caused an increase of tone without preliminary inhibition. Although serum I is an indifferent serum, it is perhaps worth while pointing out once more that its power of raising the tone of the intestine preparation is very much less than that of another indifferent serum (serum IV) from the same animal, as is shown very well in figure 28, where at 16 Ringer was replaced by serum IV. The magnification was the same as for figure 27 and the experiment was done in precisely the same way.

In figure 29 is illustrated the influence on the uterus of the sera of January 22, tested four hours after the withdrawal of the blood. The rabbit was fully grown or nearly so, but not pregnant. The uterus preparation weighed 0.33 gm. Ringer was replaced at 34 by serum II, at 37 by serum III, at 39 by serum I, and at 42 by serum IV. At 50 observation 37 was repeated.

The fact that serum III caused a much greater increase of tone of the uterus than the indifferent serum IV, while on the intestine (figures 27 and 28) serum IV had a much greater pressor effect than serum III, strengthens the inference that the smaller effect of III for the intestine was due to partial neutralization by adrenalin.

For serum II the preponderance of effect on the uterus preparation, although evident, was not so striking. It seems probable that serum II also contained some adrenalin. Nevertheless, for reasons alluded to, one would hesitate to use this observation to confirm the view that the adrenal continually gives off adrenalin.

At 44 (figure 29) a solution of adrenalin in Ringer (1 : 50,000,000) replaced the Ringer in which the uterus preparation was beating. There ensued a distinct inhibition, after which the curve rose little, if at all, beyond the initial height. At 55 adrenalin (1 : 500,000) in Ringer replaced the Ringer, and a marked increase of tone occurred, preceded by some inhibition. Yet even with this relatively strong adrenalin the increase of tone was not nearly so great as that caused in observations 37 and 50 by serum III, nor did it last nearly so long.

A purely inhibitory effect is not infrequently produced in the virgin rabbit uterus by dilute adrenalin, while stronger solutions cause the opposite effect. Figure 34 shows a still better exampie.

When the adrenalin solutions are made very dilute, a similar apparent reversal of the adrenalin action is sometimes seen with the rabbit intestine, as has been recently pointed out by Hoskins.[13] An instance is given in figure 29, where at 15 adrenalin 1 : 50,000, 000 replaced Ringer. The tone was increased and also the strength of the beats, but they diminished after washing (16). That this effect was not due merely to the replacement of Ringer's solution, in which the preparation had long been beating, by a fresh solution was shown by control experiments. A slight, transient increase of tone and of the beats may be caused by changing the Ringer, but these changes are less decided than with the adrenalin solution.

Experiment of January 19, 1912.—At 9:29 A. M. a dog weighing 7.9 kilos was etherized and carotid and tracheal cannulæ were inserted. The left great splanchnic nerve was exposed, and a cannula inserted into the transverse lumbar vein just external to the adrenal gland. A ligature was then applied loosely on this vein between the gland and the vena cava. An attempt was at once made to collect a sample of blood from the vein by connecting a small syringe with the cannula, meanwhile tightening the ligature between the adrenal and the vena cava. However, the blood clotted and the syringe was removed. The blood was then allowed to gather in the pocket of peritoneum and was sucked up, with a pipette, as quickly as it collected (sample A). It was observed particularly that the blood did not come into contact with the adrenal. Then the splanchnic was stimulated while a blood pressure tracing was being taken from the carotid. The stimulation was quite effective with regard to blood pressure. During stimulation, blood was collected partly directly from the vein, partly from the peritoneal pocket (sample B), care being taken to prevent it from coming into contact with the gland. This method of collection was not considered satisfactory, but as it had been used by others, it was thought better to continue the experiment in this way rather than to abandon it. In the other experiments, blood was collected from a cannula. Within two hours intestine and uterus tests were begun. The rabbit was a young adult in early pregnancy. The intestine preparation used for figure 30 weighed 0.42 gm. Two uterus preparations weighing respectively 1.85 gm. and 1.51 gm. were employed.

[13] R. G. Hoskins, *Am. Jour. Physiol.*, 1912, xxix, 363.

In figure 30 at 4 serum B, and at 7 serum A replaced Ringer. In both cases there was some initial inhibition followed by a moderate increase of tone. At 10 adrenalin in Ringer (1 : 50,000,000) replaced Ringer, causing a slight diminution of tone. At 18 adrenalin in Ringer (1 : 500,000) replaced Ringer and produced marked inhibition. On the uterus preparation both sera caused very decided increase of tone. Accordingly, the rather moderate increase produced in the intestine does not indicate any deficiency in the normal pressor power for smooth muscular structures, and this is consistent with the conclusion that in the intestine observations the pressor effect is partly masked by adrenalin. But still less than that of January 22 does this experiment yield decisive evidence of adrenalin blood from the gland in the absence of splanchnic stimulation.

ADRENALIN IN PATHOLOGICAL SERA.

As regards the clinical cases, no lengthy description is necessary, for in no instance was there any indication of adrenalin. The cases included:

A. A man, aged 21 years, with acute cardiorenal disease. Systolic pressure 174.

B. A man, about 30 years old, with Ménière's disease associated with lues, and with chronic tuberculosis of the lungs.

C. A woman with lues who had a much enlarged liver and marked jaundice. The serum was tinged with bile pigment and gave a Gmelin reaction.

D. A man with aortic insufficiency, greatly thickened brachial, forearm, and femoral arteries, a systolic pressure of 190 in the femoral, and 140 in the brachial, and a diastolic of 90 in brachial and femoral, in the horizontal position.

E. A woman with general paresis associated, at the time the blood was obtained, with acute mental disturbance. The blood was drawn during a veritable emotional storm, a condition supposed to be accompanied by liberation of adrenalin.

F. A patient with luetic periostitis.

G. A mulatto man, aged 42 years, with Graves' disease.[14] The symptoms were typical. The diagnosis of Graves' disease was made independently by Drs. Hoover, Phillips, and Marine. The systolic blood pressure was 170, the diastolic 90. The man complained of nervousness and general muscular weakness. He had been suffering for nine months, and had been getting progressively weaker. On examination there was no emaciation. The eyes were distinctly prominent, the pupils were dilated, and the heart rate averaged 120 over a period of four days. There was slight thyroid enlargement (symmetrical), distinct tremor, marked muscular weakness without corresponding loss of muscle volume, capillary and venous pulse in hand, very dry and brittle hair, and a trace of albumen in the urine. Slight enlargement of tonsils, and distinct substernal dullness occupying region of thymus. Splenic area of dullness increased, but spleen not palpable.

P. An elderly woman in whom a diagnosis of myxedema was made.

[14] This case is described in some detail, as Graves' disease is the condition *par excellence* in which writers on this subject claim to have found adrenalin in the blood. The patient was observed at Lakeside Hospital, to the staff of which and especially to that of the City Hospital, I am much indebted.

H. A woman, 51 years old, with a systolic blood pressure of 290, aortitis, and enlarged heart,—probably with chronic myocarditis and slight chronic interstitial nephritis. The Wassermann reaction was positive.

I. A patient with cerebral apoplexy and a systolic blood pressure of 190.

P. An elderly woman in whom a provisional diagnosis of myxedema was made.

S. A man suffering from an acute attack of gout.

V. A man, aged 26 years, with Vincent's angina but otherwise normal.

W. A woman with pneumonia. The Wassermann reaction was positive.

At 5, figure 31, serum obtained by venesection from case A, replaced Ringer. What appears to be an inhibitory effect preceded the rise of tone. The curve is reproduced to illustrate the point that mere inspection of a single curve like this is not convincing with regard to the detection of adrenalin in serum. For the serum at the date of this experiment was already five days old and if adrenalin in detectable amounts still persisted, the original adrenalin content must have been very great. Yet, when tested two days before, there had been no trace of adrenalin reaction. It is probable that the dip after 5 is due to some variation in the activity of the preparation unconnected with the addition of the serum as such, possibly to the momentary contact of a liquid as yet relatively poor in oxygen. That the preparation at this point was very sensitive to changes in the oxygen supply was shown by the fact that an increase of oxygen at 6 coincided with a very marked fall in the curve, which might have easily been mistaken for an adrenalin effect if it had occurred when the oxygen supply was not being controlled. Then the tone rapidly increased, to a maximum much above that obtained before 6.

The pressor effect of this serum on the intestine was still conspicuous ten days after the blood was drawn, but then there was no trace of an effect like that of adrenalin. At 27 (figure 31) an adrenalin solution in Ringer (1:1,000,000) replaced Ringer and caused pronounced inhibition. At 33 the serum replaced Ringer in contact with the uterus preparation. The increase of tone was at least as great as that caused by adrenalin in Ringer (1:500,000) when it replaced Ringer at 38. This, let it be repeated, merely shows that the serum preserved considerable pressor power for smooth muscle.

Figure 32 illustrates the reaction of one and the same intestine preparation to three pathological sera. As usual in each observation

the segment was initially beating in Ringer. At 2 this was displaced by serum from C. This serum was especially interesting since it was obviously rich in bile constituents. Yet its action on the intestine preparation differed in no way from that of any other serum from the peripheral circulation. At 9 Ringer was replaced by B's serum, at 11 by P's. There is no suggestion in any of the curves of adrenalin action. The effect of a 1 : 10,000,000 solution of adrenalin in Ringer is shown at 4, and that of a 1 : 15,000,000 solution at 14.

Figure 33 shows uterus reactions with the same three sera. The rabbit was nearly full grown and not pregnant. At 19 C's serum replaced one half of the Ringer. At 21 C's serum replaced all the Ringer. In both observations the serum caused decided inhibition with diminution of tone. The effect was distinctly greater with the larger dose of serum, but the curve returned sooner to its original level or even a little above it. On washing with Ringer, the curve fell somewhat (22). At 23 B's serum, and at 25 P's serum, replaced Ringer completely, and at 26 the preparation was again beating in Ringer after washing. On this uterus preparation, then, all three sera caused distinct inhibition followed by restoration of tone to normal or above and by some quickening of the beats. The effect of adrenalin on the same preparation is shown in figure 34. At 28 Ringer was replaced by 1 : 50,000,000 adrenalin in Ringer, at 29 by 1 : 15,000,000, and at 33 by 1 : 1,000,000.

This is a striking example of the opposite effects caused on the uterus by strong and weak solutions of adrenalin, the weaker solutions producing a certain amount of inhibition, and the strong solution (1 : 1,000,000) a great increase of tone with no preceding inhibition. It is certainly impossible from the reactions illustrated to deduce the presence of adrenalin in any of these sera.

Figure 35 illustrates the intestine reaction with serum from S. At 2 Ringer was replaced by the serum. At 4 after the preparation had been washed, the same amount of serum was again added. The tone increased, but diminished again much sooner than in observation 2. The cause of this was deficient oxygenation, as the cylinder became exhausted. At 5 the oxygen was increased from a fresh cylinder and the curve promptly rose, eventually to the height shown in 6. An interval of three minutes before 6 is omitted.

Had the cause of the small rise and rapid fall in observation 4 not been ascertained, and had this been the only observation with the serum, it might have been put down as a serum rich in adrenalin. When observation 17 was made, the same preparation was contracting with great regularity in Ringer. At 17 the Ringer was replaced by the serum. The beats were strengthened but no change in tone occurred. At this time the tone of the preparation was apparently incapable of being altered by any of the sera added or by adrenalin, while the force of the beats could still be markedly affected, as shown in figure 36. This, a rather unusual condition in our experience, is worthy of note apart from its bearing on adrenalin testing, where, if not taken account of, it might lead to fallacy.

In figure 36, with the preparation of figure 35, Ringer was replaced at 9 by D's serum, at 15 by E's, at 12 by adrenalin in Ringer (1 : 5,000,000), and this at 13 by adrenalin in Ringer (1 : 2,500,000). The beats became smaller for a time under the influence of the adrenalin solutions without change of tone, the exact opposite of the effect produced by the sera. It is interesting to note that at 7, before the preparation had lost the power of altering its tone, the replacement of Ringer by adrenalin in Ringer (1 : 5,000,000) caused a marked diminution of tone. There is no evidence from any of these curves that the sera in question contained adrenalin in sufficient amount to be detected. The uterus tests were also negative.

The last tracings we shall refer to are in figure 37, illustrating intestine tests of serum from G. In addition to testing the effect of the serum when it replaced Ringer directly, it was compared with such other human sera as were available by causing it to replace these. At 2 Ringer was replaced by serum from case V, and this at 3 by serum from G. A further increase of tone occurred, with no preliminary inhibition whatever. Exactly the same result was seen when at 6 the serum of G replaced that of W, which was substituted for the Ringer at 5. At 21 Ringer was replaced directly by the serum of G. The curve rose abruptly without trace of inhibition and then more slowly to the height indicated by 22. Uterus tests were equally negative.

RESULTS.

1. Tested with segments of rabbit intestine and uterus, none of the pathological sera yielded evidence of the presence of adrenalin.

2. Serum from the adrenal veins of the dog collected during massage of the gland gave a distinctly positive reaction, while serum previously collected with the minimum of mechanical and circulatory disturbance of the gland gave a negative reaction.

3. Serum from the adrenal veins without precautions to avoid disturbing the gland sometimes gave positive, sometimes negative tests.

4. Serum from blood obtained without the least disturbance of the glands, from a pocket of the inferior vena cava which received only blood from the adrenals and from the transverse lumbar veins crossing them, gave no evidence of adrenalin except during stimulation of the splanchnics when the reactions for adrenalin were positive.

5. Serum of blood collected from the vena cava of dogs by a catheter pushed up through one femoral vein until the orifice was just anterior to the openings of the adrenal veins, yielded no evidence of the presence of adrenalin whether the splanchnics (one or both) were stimulated or not, any adrenalin contributed by the glands during stimulation of the splanchnics being too much diluted.

EXPLANATION OF PLATES.

All the tracings are to be read from left to right. Time is marked in half minutes. At the beginning of each observation, unless otherwise stated, the preparations were always beating in Ringer's solution, serum or adrenalin solution, if previously employed, having been carefully washed away with Ringer. Except when otherwise mentioned, the various sera were tested as soon as they could be separated from the blood, as a rule within three or four hours. The larger apparatus, holding 4.5 c.c., was used for the observations illustrated in figures 3 to 12, and in figure 31; the smaller, holding 2.5 c.c., was used for the rest. Unless a statement is made to the contrary, the liquid used for any particular observation was sufficient to replace completely that in which the preparation was initially beating. The tracings have all been reduced one half.

PLATE 77.

FIG. 1. Intestine segment beating in Ringer. At 6 the oxygen was increased.

FIG. 2. Intestine contracting in Ringer. At 33, 1 c.c. of serum was introduced. At 34 an additional 2 c.c. of the same serum was added.

FIG. 3. Intestine. At 31 adrenalin solution 1 : 10,000,000 in calf serum was added. At 33 adrenalin 1 : 10,000,000 in Ringer replaced Ringer.

FIG. 4. The same preparation that was used for figure 3. At 29 adrenalin 1 : 5,000,000 in the calf serum replaced Ringer.

FIG. 5. The same preparation that was used for figure 4. At 25 calf serum, at 23 and 27 adrenalin 1 : 25,000,000 in the calf serum, and at 20 adrenalin solution 1 : 25,000,000 in Ringer replaced Ringer.

FIG. 6. The same preparation that was used for figures 3 to 5. At 35 adrenalin 1 : 50,000,000 in the calf serum, at 37 the serum itself, and at 39 adrenalin in Ringer 1 : 50,000,000 replaced Ringer.

FIG. 7. Intestine. A fresh preparation from the rabbit used for figures 3 to 6. At 4 calf serum, at 7 adrenalin 1 : 5,000,000 in the serum, at 9 adrenalin 1 : 5,000,000 in Ringer, and at 11 adrenalin 1 : 5,000,000 in the serum replaced Ringer.

FIG. 8. Uterus. At 5 adrenalin 1 : 25,000,000 in calf serum, and at 7 adrenalin 1 : 25,000,000 in Ringer replaced Ringer. At 6 the oxygen was increased.

FIG. 9. The same preparation that was used for figure 8. At 10 adrenalin solution 1 : 50,000,000 in calf serum, and at 12 adrenalin solution 1 : 50,000,000 in Ringer replaced Ringer.

FIG. 1.

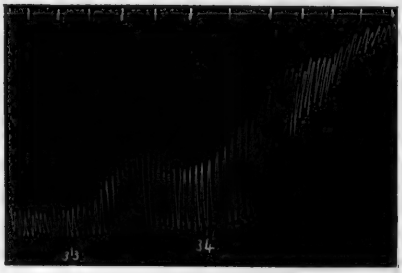


FIG. 2.

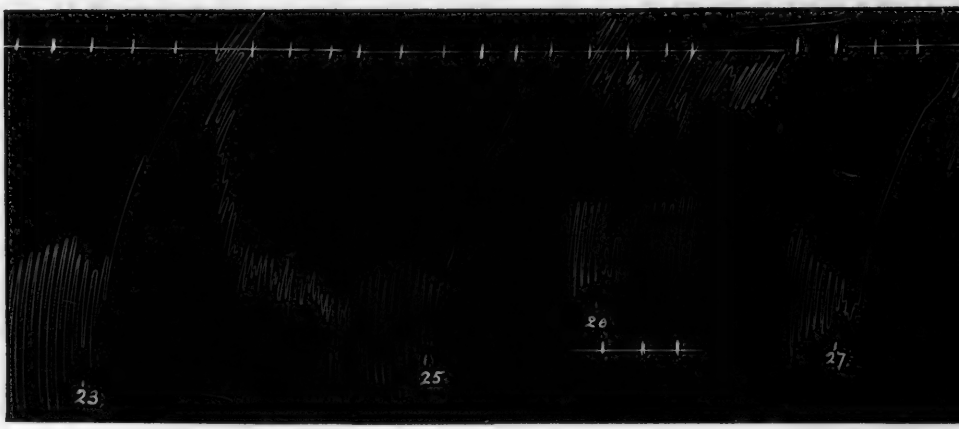
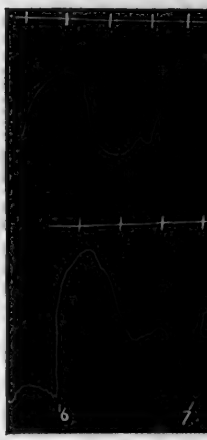


FIG. 5.



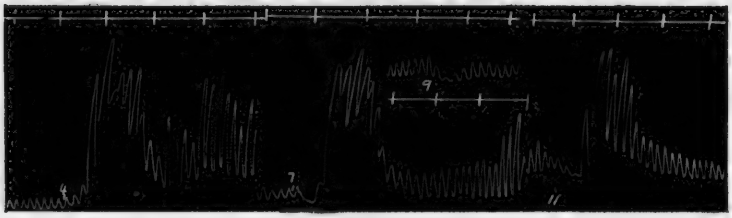


FIG. 7.

FIG. 3.

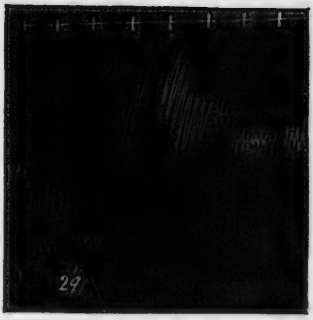


FIG. 4.

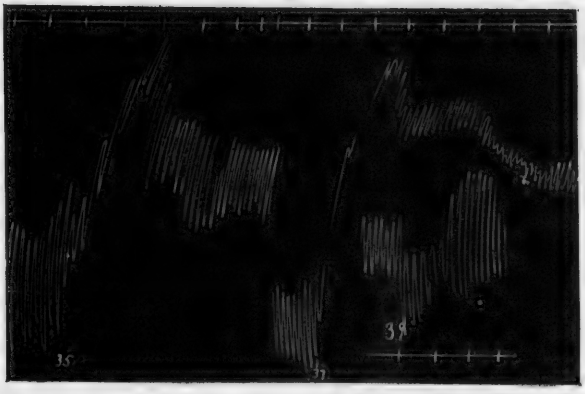


FIG. 6.

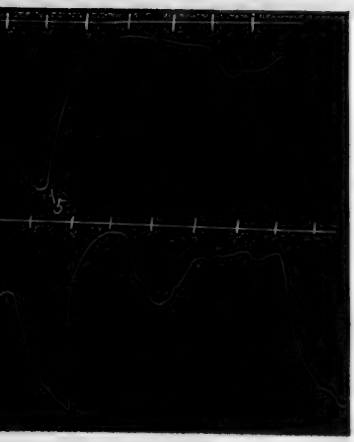


FIG. 8.

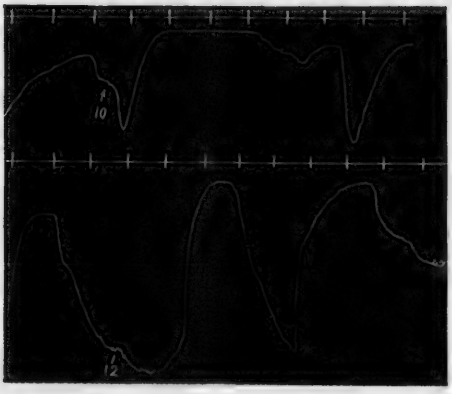


FIG. 9.

PLATE 78.

FIG. 10. The same preparation as in figure 8. At 14 adrenalin in calf serum 1 : 10,000,000, and at 16 adrenalin in Ringer 1 : 10,000,000 replaced Ringer.

FIG. 11. The same uterus preparation that was used in figures 8 to 10. At 18 adrenalin in calf serum 1 : 5,000,000, and at 21 adrenalin in Ringer 1 : 5,000,000 replaced Ringer.

FIG. 12. The same uterus preparation as in figure 11. At 29 adrenalin 1 : 50,000 in serum, and at 34 adrenalin 1 : 50,000 in Ringer replaced Ringer.

FIG. 13. Intestine. Observations 2, 4, and 9 show the effects produced by the replacement of Ringer by serum I, serum II, and serum IV, respectively, of February 13. At 11 adrenalin 1 : 25,000,000 in serum IV, at 13 adrenalin 1 : 12,500,000 in serum IV, and at 15 adrenalin in Ringer 1 : 12,500,000 replaced Ringer.

FIG. 14. The same intestine preparation as in figure 13. At 6 serum III of February 13 replaced Ringer.

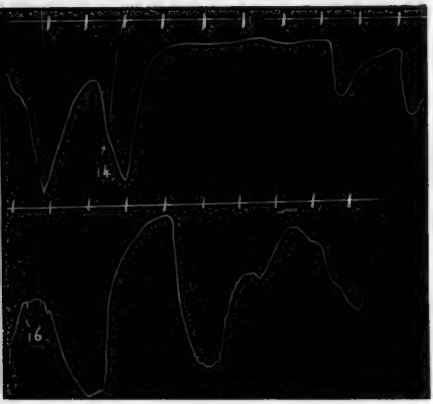
FIG. 15. The same preparation as in figure 14. At 17 adrenalin in serum IV of February 13, 1 : 1,250,000, and at 19 adrenalin in Ringer 1 : 1,250,000 replaced Ringer.

FIG. 16. A fresh intestine preparation. At 28 serum IV of February 13 replaced Ringer. At 29 serum III replaced serum IV. At 36 serum IV replaced Ringer, and adrenalin 1 : 12,500,000 in serum IV replaced serum IV at 37. At 40 Ringer was replaced by serum IV, and this at 41 by adrenalin 1 : 500,000 in serum IV.

FIG. 17. Intestine preparation. At 20 Ringer was replaced by serum IV of February 14, and this at 21 by serum III of February 14. At 22 serum III was displaced by serum IV. At 24 serum IV replaced Ringer. At 25 serum IV was replaced by serum II. At 28 Ringer was replaced by serum IV, and this at 29 by adrenalin 1 : 1,000,000 in serum IV. After washing, adrenalin in Ringer 1 : 1,000,000 replaced Ringer at 40.

FIG. 18. Uterus preparation. At 42 Ringer was replaced by serum IV of February 14, and this at 43 by serum III. At 46 serum IV was added to the preparation after washing. At 47 serum IV was replaced by serum II. After washing, serum IV was again introduced at 50, and was replaced at 51 by adrenalin 1 : 1,000,000 in serum IV.

FIG. 19. Intestine. Serum III and serum IV of February 14, after being kept for forty-eight hours in the ice chest. At 52 Ringer was replaced by serum IV, and this at 53 by serum III. At 55 Ringer was replaced by serum IV, and this at 56 by adrenalin 1 : 5,000,000 in serum IV. At 57 adrenalin 1 : 500,000 in serum IV replaced the 1 : 5,000,000 solution. At 59 Ringer was replaced by serum IV, and this at 60 by adrenalin 1 : 1,000,000 in serum IV.



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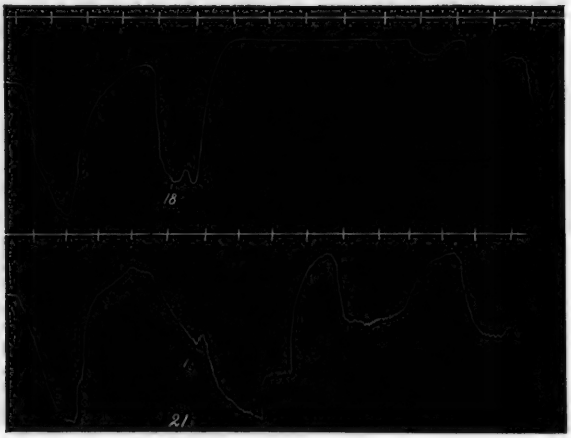
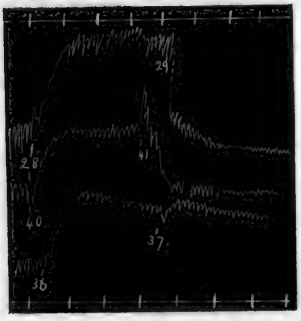


FIG. 11.

FIG. 15.



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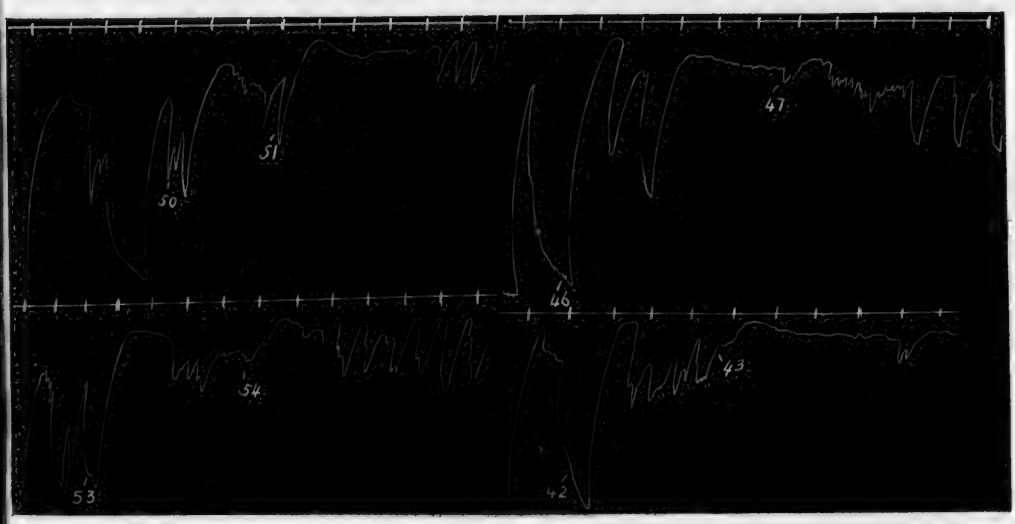

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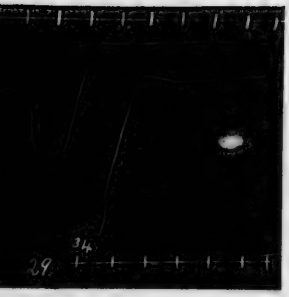
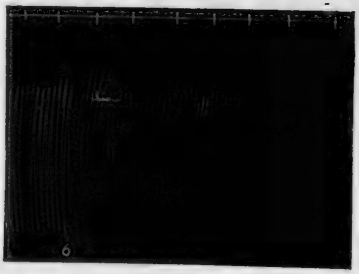


FIG. 12.

FIG. 13.



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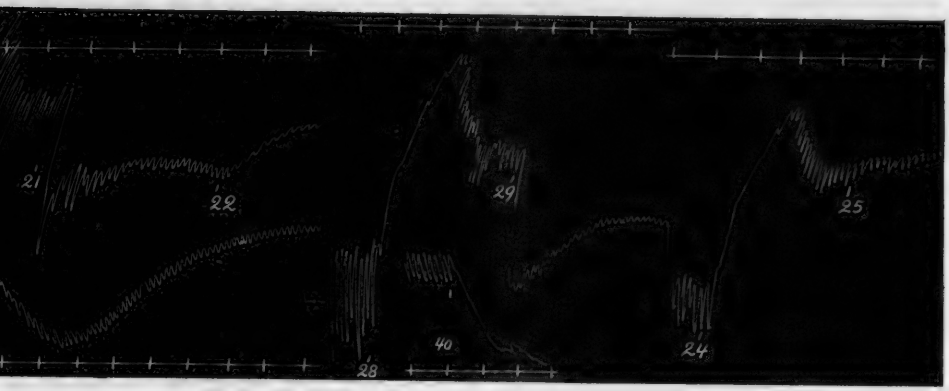

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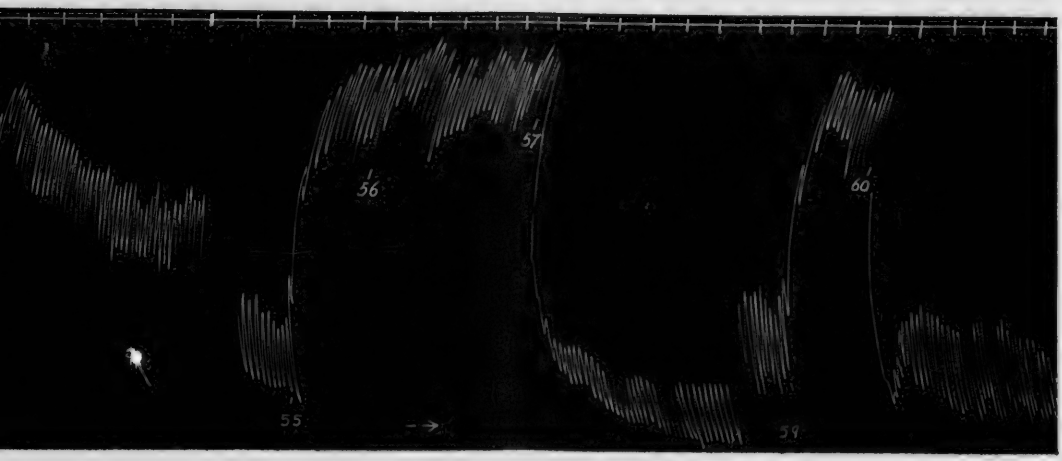


FIG. 19.

PLATE 79.

FIG. 20. Uterus preparation. At 25 Ringer was replaced by serum V of February 16 (drawn twenty-four hours before), and this at 26 by adrenalin 1 : 5,000,000 in serum V. At 28 Ringer was replaced by serum I of February 14 (obtained three days before), and this at 29 by serum III of February 14.

FIG. 21. The same intestine preparation as in figure 19. At 65 Ringer was replaced by serum I of February 16, a few hours after being drawn, and this at 66 by serum III of February 16. At 68 Ringer was replaced by serum I of February 16, and at 69 additional serum I was run into the cylinder. At 70 serum I was replaced by serum III. At 75 Ringer was replaced by serum II, and this at 76 by serum III.

FIG. 22. The same intestine preparation as used in figure 21. At 78 Ringer was replaced by serum VI of February 16 (a few hours after the blood was withdrawn), and this at 79 by serum V. At 49 Ringer was replaced by serum VI, and this at 50 by serum IV. At 81 Ringer was replaced by serum VI, and this at 82 by serum III of February 16.

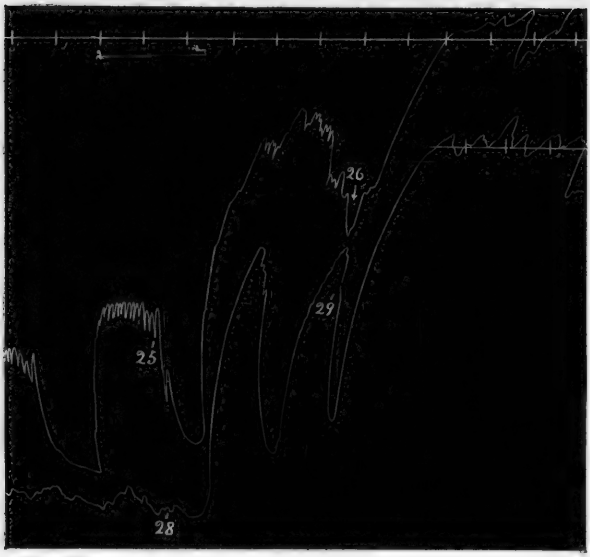
FIG. 23. Uterus preparation. At 84 Ringer was replaced by serum II of February 16 (a few hours after withdrawal), and this at 85 by serum III. At 92 Ringer was replaced by serum VI, and this at 93 by adrenalin 1 : 1,000,000 in serum VI.

FIG. 24. Uterus. At 9 serum II of February 13 (obtained four days previously), and at 10 serum III of February 13 replaced Ringer. At 2 Ringer was replaced by serum VI of February 16, and this at 3 by serum III of February 16. At 15 Ringer was replaced by serum VI, and this at 16 by adrenalin 1 : 1,000,000 in serum VI. At 22 Ringer was replaced by serum V, and this at 23 by serum III of February 16.

FIG. 25. The same uterus preparation that was used for figure 24. At 5 Ringer was replaced by diluted serum VI of February 16, and this at 6 by diluted serum III of February 16, which at 7 was replaced by diluted serum VI.

FIG. 26. Intestine. At 2 Ringer was replaced by serum VI of January 22, at 4 by serum V, at 6 by serum III of January 22, and at 8 by serum III.

FIG. 27. The same intestine preparation that was used for figure 26. At 10 Ringer was replaced by serum II of January 22. After washing, the experiment was exactly repeated at 12. At 14 Ringer was replaced by serum I of January 22.



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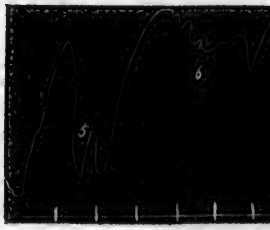

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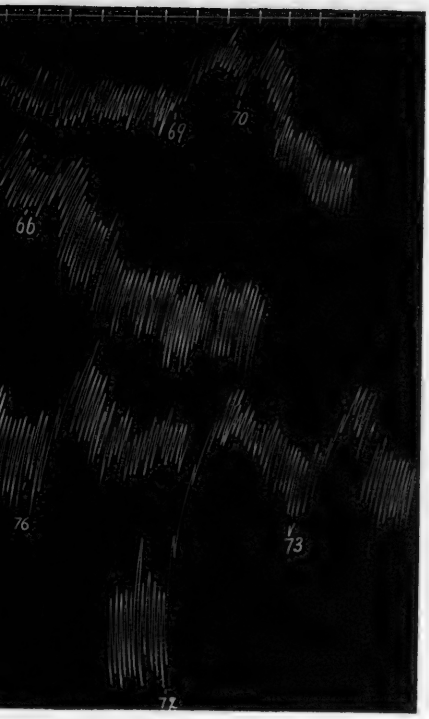

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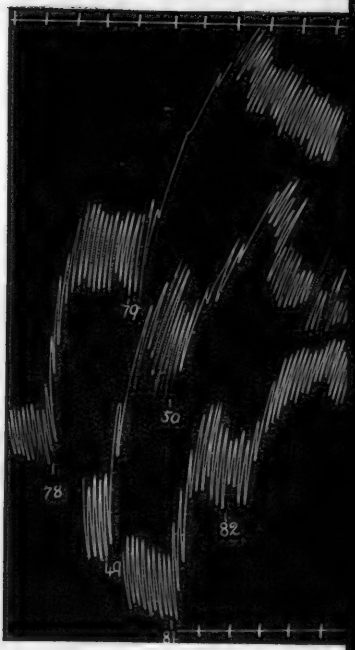

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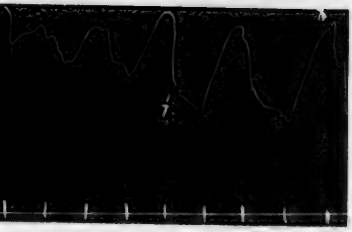

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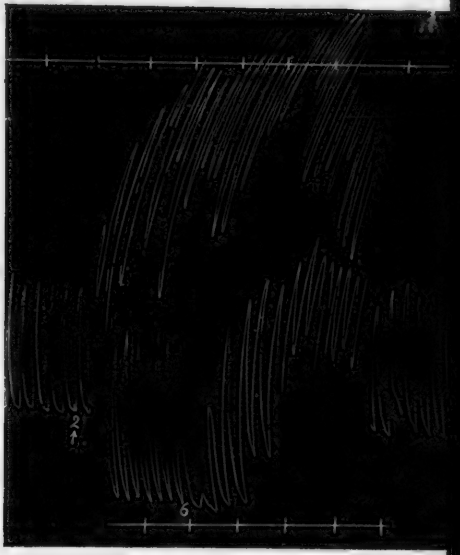

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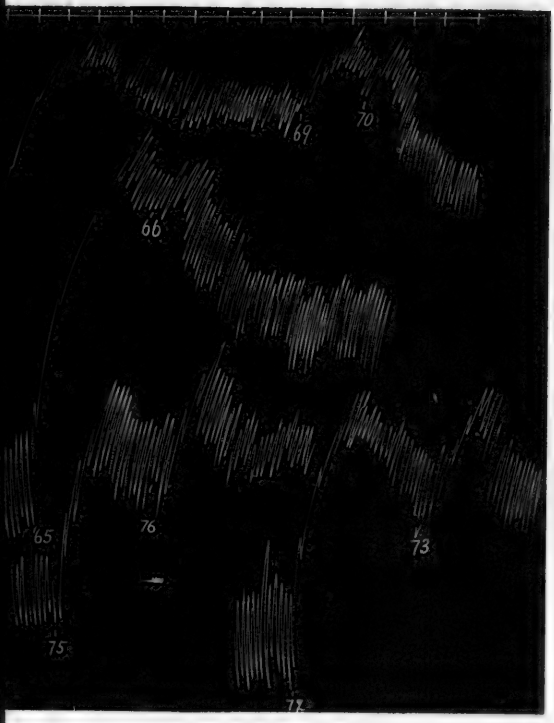
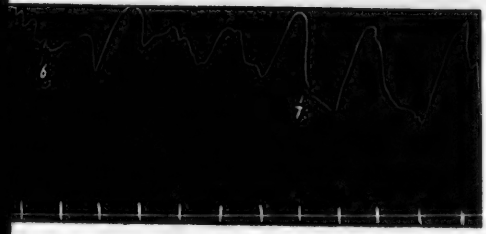


FIG. 21.

FIG. 22.



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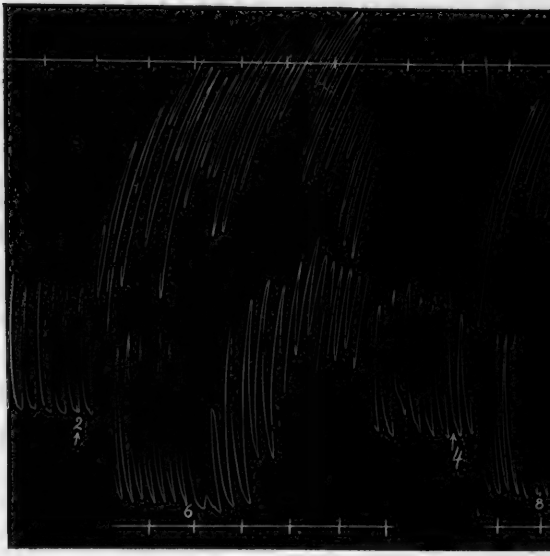

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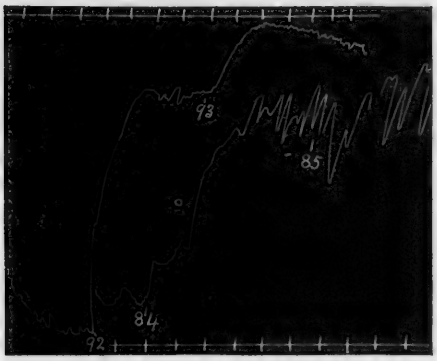

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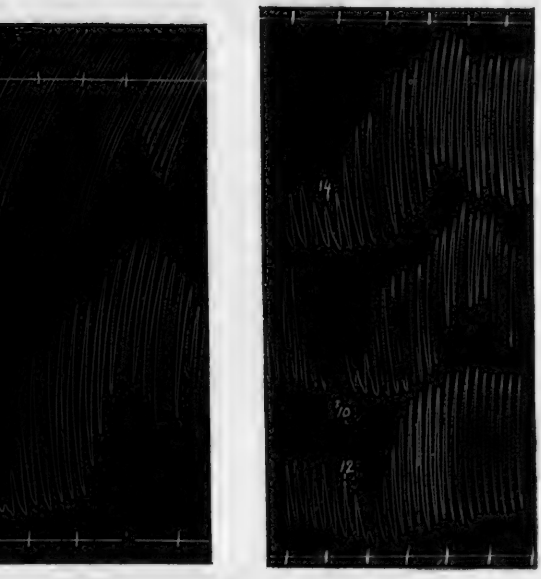


FIG. 27.

PLATE 80.

FIG. 28. The same intestine preparation as that used for figure 27. At 16 Ringer was replaced by serum IV of January 22.

FIG. 29. Intestine. At 15 Ringer was replaced by adrenalin 1 : 50,000,000 in Ringer, and at 16 the preparation was again beating in Ringer.

Uterus preparation. Sera of January 22 were tested four hours after withdrawal of blood. At 34 Ringer was replaced by serum II, at 37 by serum III, at 39 by serum I, and at 42 by serum IV (the very small elevations on the curve are due to oxygen bubbles). At 50 Ringer was replaced by serum III, at 44 by adrenalin 1 : 50,000,000 in Ringer, and at 55 by adrenalin 1 : 500,000 in Ringer.

FIG. 30. Intestine. At 4 Ringer was replaced by serum B of January 19, at 7 by serum A of January 19, at 10 by adrenalin 1 : 50,000,000 in Ringer, and at 18 by adrenalin 1 : 500,000 in Ringer.

FIG. 31. Intestine. At 5 Ringer was replaced by serum from case A, and at 6 the oxygen supply was increased. At 27 Ringer was replaced by adrenalin 1 : 1,000,000 in Ringer.

Uterus preparation. At 33 Ringer was replaced by serum from case A, at 38 Ringer was replaced by adrenalin 1 : 500,000 in Ringer.

FIG. 32. Intestine. At 2 Ringer was replaced by serum from case C, at 9 by serum from case B, at 11 by serum from case P, at 4 by adrenalin 1 : 10,000,000 in Ringer, and at 14 by adrenalin 1 : 15,000,000 in Ringer.

FIG. 33. Uterus. At 19 half of the Ringer was replaced by the serum of case C. At 21 Ringer was completely replaced by the serum of C, at 23 by serum from case B, and at 25 by serum from case P. At 26 the preparation was beating in Ringer after washing.

FIG. 34. The same uterus preparation as that used for figure 33. At 28 Ringer was replaced by adrenalin 1 : 50,000,000 in Ringer, at 29 by adrenalin 1 : 15,000,000, and at 33 by adrenalin 1 : 1,000,000 in Ringer.

FIG. 35. Intestine. At 2 Ringer was replaced by serum from case S. At 4 after washing, Ringer was again replaced by the serum from S. At 17 Ringer was replaced by serum from S. Note that the tone is now unaltered. The lower time-trace belongs to observation 17.

FIG. 36. At 9 Ringer was replaced by serum from case D, at 15 by serum from case E, and at 12 by adrenalin 1 : 5,000,000 in Ringer, which at 13 was replaced by adrenalin 1 : 2,500,000 in Ringer. At 7, before the preparation had lost the power of altering its tone, Ringer was replaced by adrenalin in Ringer 1 : 5,000,000.

FIG. 37. Intestine. At 2 Ringer was replaced by serum from case V, and this at 3 by serum from case G. At 5 Ringer was replaced by serum from case W, and this at 6 by serum from case G. At 21 Ringer was replaced by serum from case G; 22 in the continuation of the curve. The lower time-trace belongs to observations 21 and 22.

FIG. 28.

FIG. 29.

FIG. 34.

FIG. 30.

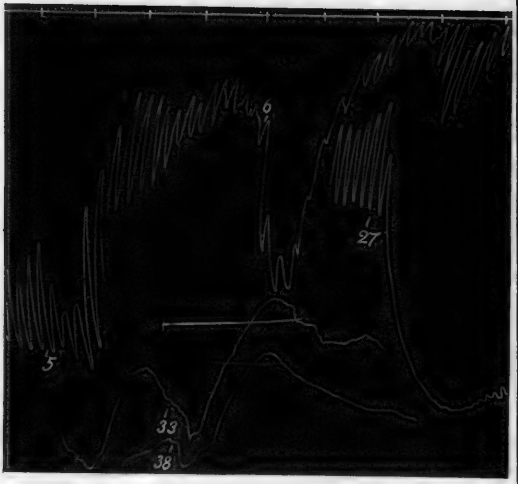
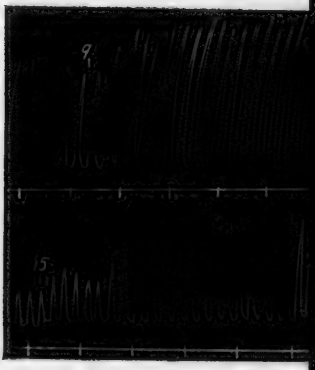


FIG. 31.

FIG. 35.



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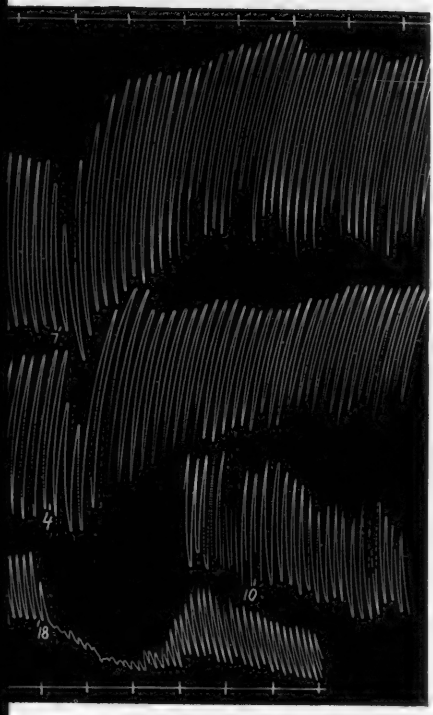

FIG. 30.

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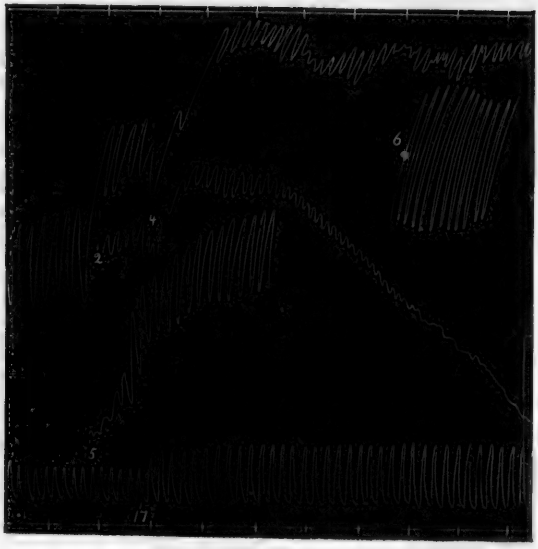

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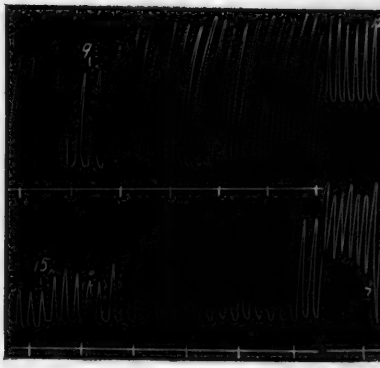


FIG. 36.

FIG. 32.

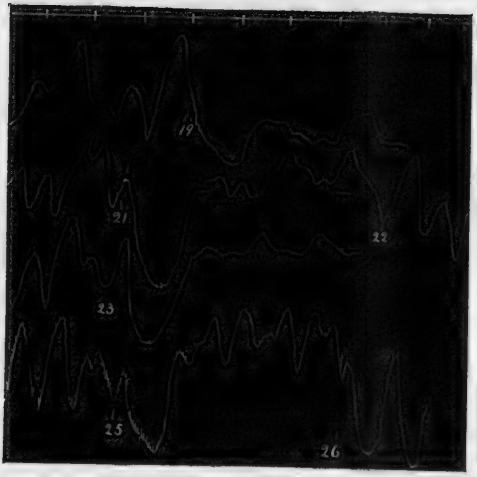


FIG. 33.

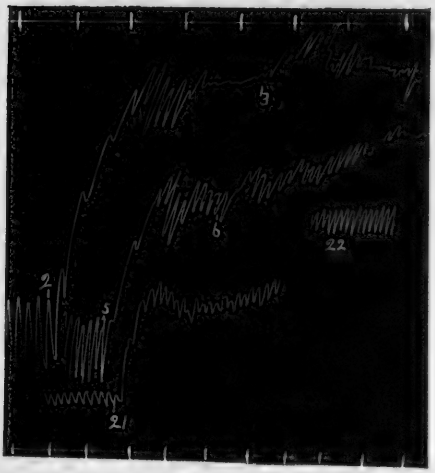


FIG. 37.

MALARIAL PIGMENT (HEMATIN) AS A FACTOR IN THE PRODUCTION OF THE MALARIAL PAROXYSM.*

By WADE H. BROWN, M.D.

(*From the Pathological Laboratory of the University of North Carolina, Chapel Hill, N. C.*[1])

It is remarkable that, from the enormous amount of investigation that has centered about the malarial parasite and its relation to malarial fevers, there has come no clear exposition of the mode of production of the various phenomena of the malarial paroxysm. It is true that these phenomena have been ascribed to the presence in the circulation of some toxic substance, or substances, elaborated by the malarial parasite, and that the blood at the time of the segmentation of the parasite has been shown to possess toxic properties. Still, as far as I know, the nature of these toxic agents remains unknown, as no one has clearly demonstrated the presence of any definite substance, which, when introduced into the circulation, could reproduce the symptom complex of the malarial paroxysm. The observations embodied in this report are offered, therefore, with the hope of shedding some light upon the question.

In the course of some work on hematin metabolism, it was noted that a rabbit that had received an intravenous injection of alkaline hematin developed a very pronounced shaking chill, strikingly like that of malaria. As the author has attempted to show in a previous paper,[2] the pigment elaborated from the hemoglobin of the red blood corpuscle by the malarial parasite and liberated into the circulation of the host at the time of segmentation of the parasite is undoubtedly hematin. It at once appeared possible, therefore, that we might find in this substance one of the hypothetical toxins operative in malaria.

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, March 29, 1912.

[1] The first seven experiments of this investigation were done in the Pathological Laboratory of the University of Wisconsin, Madison, Wis.

[2] W. H. Brown, *Jour. Exper. Med.*, 1911, xiii, 290.

The investigation of this question now embraces a series of ninety observations upon the effect of the intravenous injection of alkaline hematin, eighteen rabbits having been used for test purposes.

TECHNIQUE.

Materials Used and Their Preparation.—The hematin used was derived from three sources, rabbit blood, dog blood, and ox blood, but in all cases it was prepared by the Schalfijew process. The solutions for injection were made in 0.85 per cent. salt solution containing 1.5 or 2 per cent. bicarbonate of sodium. The strength of the hematin employed has varied from 1.5 to 5 milligrams per cubic centimeter. The hematin was added to the sterile solvent and heated to 100° C. for five to ten minutes, and was then allowed to stand for twelve to twenty-four hours, when it was again heated and, while still hot, filtered into sterile flasks. The subsequent treatment of these solutions has varied somewhat. Solutions prepared thus, and again heated to 100° C. for five to ten minutes have apparently remained sterile. In a few instances, however, the filtered solution has been autoclaved to insure sterility.

It has been found extremely difficult to prepare hematin solutions of absolutely uniform character. With a given preparation of hematin and with the same technique in the preparation of the solutions, two distinct types of solution may be obtained: one, a perfectly clear, deeply colored solution that even under the microscope shows very few particles of pigment suspension, and on standing shows no precipitation in the flask; the other, a turbid solution that appears chocolate brown in thin layers and under the microscope shows myriads of pigmented particles and droplets floating in an only faintly colored fluid. Much of this pigment is precipitated on allowing the solution to stand. This latter "colloidal" type of solution or suspension, manifests all the optical properties of an alkaline hematin solution and the suspended pigment readily passes through Schleicher and Schüll filter paper No. 597 which has been used as the routine filter. With different preparations of hematin these variations in solubility are continually appearing. Reference is made to this feature of the hematin solution to indicate the

difficulty in maintaining absolutely uniform experimental conditions and accurate dosage.

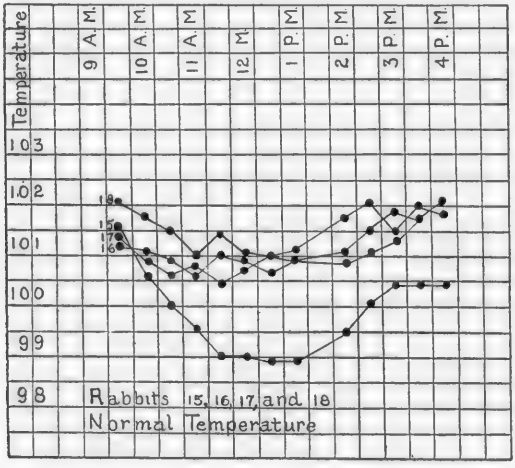
Experimental Procedure.—Briefly, the experiments have been conducted according to the following plan: The animals were accustomed to laboratory surroundings by being kept in cages from one to two days before any observations were made. The normal temperature curve of each animal was then established by rectal temperature, taken every half hour from 8 or 9 A. M. until 3 or 4 P. M., the time to be covered by subsequent experiments. As it seemed probable that the sodium bicarbonate salt solution used as the solvent for hematin might produce some toxic manifestations when injected intravenously, the action of this solution was determined in a number of instances, usually in such doses as were subsequently to be administered with the hematin. While these control tests were usually made prior to the injection of hematin, some such tests followed the administration of hematin. The animals were next given intravenous injections of alkaline hematin at 8 or 9 A. M., and the resulting phenomena were noted, the temperature being taken as previously, although fifteen minute observations were frequently made.

Dose of Hematin.—It is evident that the question of dosage in such a series of experiments is one of prime importance. To carry out the object of these experiments it is quite essential that the dose of hematin employed be somewhat comparable to that liberated into the human circulation at the time of segmentation of a generation of parasites. While there is no evidence to show that all the hemoglobin of the infected red corpuscles is decomposed to form hematin, if we may assume that such is the case, and, further, that a 1 to 5 per cent. infection of red blood corpuscles is not uncommon, we have sufficient data upon which to base a calculation of the approximate amount of hematin liberated into the human host with the segmentation of a given generation of parasites. Basing our calculations on the presence of 8.5 grams of hemoglobin per kilo of body weight, and 4.47 per cent. of hematin in hemoglobin,[3] we find that in a 1 per cent. infection of the red blood corpuscles approximately

[3] Olof Hammarsten, A Text Book of Physiological Chemistry, translated by Mandel, New York, 1901, p. 139. These figures are probably high.

3.7 milligrams of hematin per kilo of body weight would be liberated. In my experiments the dosage has varied from 1.3 milligrams to 28 milligrams per kilo of body weight, given in single or in divided doses, corresponding roughly to an infection of 0.3 to 7.5 per cent. of the red blood corpuscles.

Normal Temperature of the Rabbit.—In undertaking an investigation which must necessarily deal so largely with variations in the temperature of the experimental animal, it is imperative that the basis of comparison between normal and abnormal fluctuations of temperature be as free from objection as possible. Unfortunately, the temperature of different rabbits varies widely, even when kept under exactly the same conditions. In apparently normal animals I have found the individual extremes between 98° and 103° F. Likewise, the fluctuations of temperature in a given animal may be quite considerable, but usually follow a fairly definite course.



TEXT-FIG. 1. Normal temperature of four rabbits during the period of the experiments.

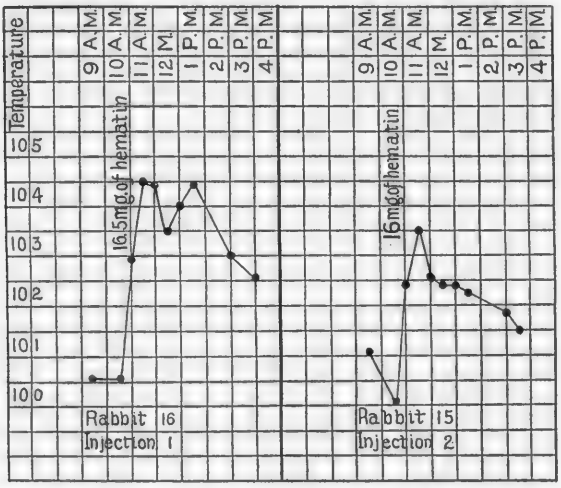
The course and fluctuations of temperature of the normal rabbit under experimental conditions, as well as the individual difference in temperatures, are shown in text-figure 1. This chart shows the normal temperatures recorded for rabbits 15, 16, 17, and 18. The

first three animals were from the same litter, about three quarters grown, and weighed 1,600 to 1,700 grams. Number 18 was full grown and weighed 1,840 grams. All the records were taken at the same time and all conditions were as nearly alike as possible. While three of these curves coincide closely, the fourth shows an extremely low and irregular curve of temperature. It should be noted that the temperature in all instances has a downward trend during the morning hours, and does not show an upward tendency until about noon, when there is a gradual rise, which ultimately reaches as high as the temperature at the first observation or even higher. This temperature curve has been fairly constant in my entire series of experiments.

Effect of Hematin upon Temperature.—If, for purposes of comparison, we adopt the classical division of the malarial paroxysm into a cold stage, a hot stage, and a stage of sweating, with the concomitant symptoms belonging to each, certain of these manifestations are capable of accurate measurements in an experimental animal, while others may be determined with a fair degree of accuracy by close observations, and still others are wholly indeterminable. Of prime importance among these phenomena of the malarial paroxysm is the question of fever.

In estimating the temperature effects, in all instances at least three facts are to be taken into consideration; the nature of the effect, the degree of the effect, and the duration of the effect. While it has been possible to assemble much of the data concerning the effects of hematin upon the temperature in an appended table which shows the abbreviated protocols of the entire series of experiments, it must be fully appreciated that such tabulations of statistics are wholly inadequate to present many features of the experiments that are equally as important as those thus presented, and attention will be especially directed to such features. Further, as can be seen from these tables, it has been the object of the author to study effects in individual animals with a variety of doses, as occasion suggested, rather than to mould all the experiments to a single type or plan, for it became evident early in this investigation that individual peculiarities of the animals played a prominent rôle in the results obtained.

Without exception, every dose of hematin administered has elicited a definite temperature response. With but three exceptions, this response has been characterized by a sharp rise in temperature, reaching the fastigium in about an hour and a quarter. The further course was somewhat variable, although in most cases with a dose of 15 milligrams, or less, per kilo, there was a rather sharp fall of temperature for thirty minutes to one hour, followed by a secondary rise of variable extent and duration or a very gradual decline requir-



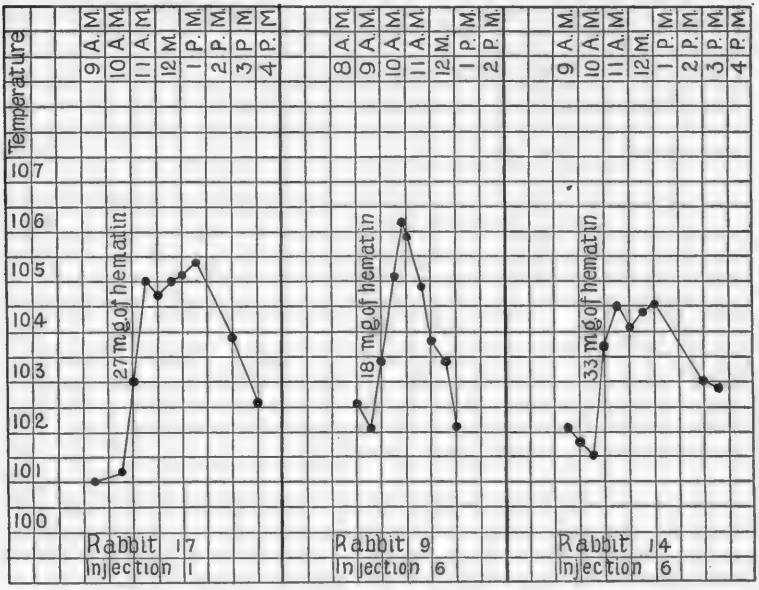
TEXT-FIG. 2. The usual types of temperature curve following injections of hematin.

ing several hours to reach normal again. Two such temperature curves are shown in text-figure 2.

These curves are subject to innumerable variations depending upon the dose, the stage of the experiment, and upon the individual peculiarities of animals. Some of the more important variations are an accentuation and prolongation of the secondary rise, usually shown with initial and large doses, or a defervescence that is almost as sharp as the rise in temperature. A third variation, which includes the three exceptions previously noted, is met with in instances

of marked intoxication and is characterized by an initial drop in temperature with a subsequent rise. All three of these modifications are illustrated in text-figure 3.

The extent of the temperature elevation is, within certain limits, proportional to the amount of hematin injected. The temperature effect, being very slight with small doses, increases with the dose,



TEXT-FIG. 3. Variations in the temperature curve following large doses of hematin or repeated injections of hematin.

until we begin to obtain signs of an over-intoxication when the elevation may be much less than with smaller doses, the optimum dose usually being between 10 and 15 milligrams per kilo of body weight. The elevation of temperature obtained with such optimum doses is from 3° to 3.5° F., and it is exceptional that a greater rise is reached. Occasionally, however, the temperature may rise 4° F. or even higher in highly susceptible animals. The greatest elevation recorded in my series of experiments was 4.9° F. in animal 9, with a dose of 18 milligrams of hematin per kilo.

In well-marked reactions, the temperature usually returns to within the normal range in the course of three to five hours. With large or initial injections of hematin, the period of elevated temperature is more prolonged than under other circumstances and seldom reaches normal in less than four hours, occasionally requiring as much as six hours. Exceptionally, the return to normal may be rapid (text-figure 3).

The method of administration also plays an important part in the results. A given dose of hematin injected in two or three fractional doses at intervals of fifteen to thirty minutes produces a much more marked elevation of temperature than when given at a single injection, an effect that is well shown in rabbit 13. This is particularly true of the smaller, or optimum doses, while with larger doses the increased potency may be manifested by a slowing of the rise of temperature, a cessation of the rise, or even a fall upon the injection of the second fraction, as illustrated in rabbit 12.

Neither the source of the hematin nor the type of solution seems to play an important part in the results that I have obtained. That is, there has been but slight difference between the action of rabbit, dog, or ox hematin, or between the action of the perfectly clear hematin solution and that containing much finely divided hematin in suspension.

However, a few tests seem to indicate that solutions of hematin gradually lose their pyrogenic properties with age or when subjected to high and prolonged temperature. This apparent decrease may be seen by comparing the results obtained in rabbit 7, injection 3, and rabbits 8, 10, and 11. Further, it can readily be imagined that all animals will not be found equally susceptible to the toxic action of hematin. A few will exhibit a marked sensitiveness and a few will be found extremely resistant, the optimum dose in the one producing but slight effect in the other. This variation in susceptibility was strikingly illustrated by animals 13 and 14 which were under observation at the same time. Rabbit 13 was a typically resistant animal, and rabbit 14 was highly sensitive.

Effect of Sodium Bicarbonate Salt Solution upon the Temperature.—Animals injected with the bicarbonate salt solution alone, for purposes of control, almost invariably showed an elevation of tem-

perature in proportion to the size of the dose, and about one third to one half the elevation produced with an equal amount of hematin solution, depending somewhat upon the concentration of the hematin in the solution. With small doses of the control medium, the fluctuations of temperature were usually within what might be termed the normal range and were such that it is difficult to say whether they are more than incidental to the process of injection. Large doses may produce a rise in temperature corresponding approximately to the over-intoxicating effect of large doses of hematin. In such instances, however, other features of the clinical picture will distinguish sharply between the two cases. In all instances, therefore, there were distinct differences between the action of the sodium bicarbonate salt solution and the action of the hematin solution, such that there can be no question as to the part played by the hematin in these experiments.

Other Phenomena of the Hematin Paroxysm.—Apart from the elevation of temperature in the experimental animal, the paroxysm of hematin intoxication presents other features which are of equal importance and show a strong resemblance to corresponding phenomena of the malarial paroxysm. For the first fifteen to twenty minutes following the injection of hematin, the rabbit usually manifests a slight degree of restlessness, then crouches in a corner of the cage. In the second stage of the paroxysm the vessels of the ears contract giving to the shaved ears a pale and cyanotic hue, while at the same time the ears become decidedly cold. In pronounced cases the surface temperature (temperature of the ears) may be more than 30° F. below the rectal temperature. The lowest temperature recorded in this series of experiments was 63.5° F. with a room temperature of 62.5° F. and a rectal temperature of 105° F. During this stage the animal's ears usually lie on its back, and the hair tends to become erect, presenting the picture of an animal that is cold. Meanwhile, the rabbit shows convulsive tremors or shivering, but rarely any continued or pronounced shaking. This stage of chill lasts from forty-five minutes to one hour, and is terminated rather abruptly by a dilation of the superficial vessels, the ears rapidly becoming flushed and hot. The animal now moves about the cage or stretches out and remains quiet. Further than this, the

third or hot stage of the paroxysm possesses no especial symptoms and its limit can be fixed only by the course of the temperature, which may remain well above normal for several hours, or sink to normal within an hour. During the third stage and the latter part of the second stage of the paroxysm the animal shows a pronounced thirst which is undoubtedly referable to the febrile condition.

The most striking and at the same time the most constant of all these symptoms are the contraction and dilatation of the superficial vessels associated with the corresponding lowering and elevation of the surface temperature.

The contrast between the symptoms of the animal injected with hematin and those of control animals is quite as sharp as in the instance of the temperatures. With doses of hematin sufficiently large to produce pronounced symptoms of the type described, corresponding doses of sodium bicarbonate salt solution produce practically no recognizable effect. There may be a suggestive or very transient change in the surface vessels and the temperature, but nothing that is definite or constant. When, however, larger doses, *e. g.,* ten cubic centimeters per kilo, are given, phenomena simulating the picture of hematin intoxication may be elicited, but the changes are not so definite or constant.

If now we correlate these symptoms with the temperature curve, we find that the elevation of temperature during the first stage is slight, and that the second stage of the paroxysm corresponds closely with the period of rising temperature, the initial drop coinciding sharply with the vascular dilatation and flushing of the ears and the elevation of the surface temperature. The third or hot stage, as previously noted, corresponds to the duration of the temperature above normal.

As in the case of the temperature, all other phenomena of hematin intoxication seem to be exaggerated when a given dose of hematin is divided into several fractional doses, the cycle of phenomena following closely consequent changes in the temperature curve.

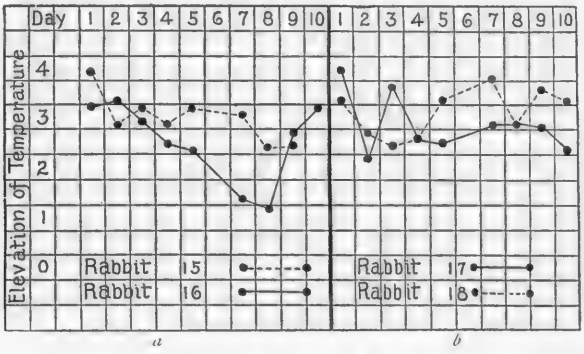
It must be pointed out, however, that the prominence of these paroxysmal phenomena and the degree of elevation of the temperature are by no means always parallel. The toxic paroxysmal phenomena may be present to a high degree in an animal that shows

only a slight elevation of temperature, and in most instances such a condition is to be regarded as evidence of over-intoxication.

Acquired Resistance.—Early in the course of these experiments it became evident that repeated injections of a given dose of hematin in the same animal did not give uniform results. The results, however, were of such a nature as to suggest that the animal acquired a certain degree of tolerance which, in turn, might be broken when the intoxication was pushed sufficiently. To determine this point the following experiment was carried out.

Experiment.—Four rabbits, weighing respectively 1,600, 1,650, 1,790, and 1,840 grams, were injected on ten successive days with a solution of ox hematin containing 5 mg. of hematin to 1 c.c. The first two animals received 10 mg. of hematin per kilo of body weight, and the other two received 15 mg. per kilo. Rectal temperatures were recorded every half hour.

The results are shown in text-figure 4. In *a* is shown the differences of elevation of temperature on successive days of the two



TEXT-FIG. 4. Variations in the elevation of the temperature with repeated injections of a given dose of hematin.

animals receiving ten milligrams per kilo, and in *b* those receiving fifteen milligrams per kilo.

While the curve of temperature reaction in each case is extremely irregular, it is in general characterized by a tendency towards a decrease, which in the instance of animals 16 and 17 persists throughout the experiment. With animals 15 and 18, however, a second phase of increased reaction is developed. These animals also

exhibited the most marked symptomatic effects throughout the experiment. If the temperature curve alone be considered, it is certain that the tendency is toward a decreasing response to successive injections of hematin and this I have found to be true in other experiments. If we take into consideration other evidence of intoxication, however, as in animals 15 and 18, this decrease seems referable not so much to a tolerance as to over-intoxication. Again, as these symptoms of intoxication decrease and the fluctuations of temperature increase correspondingly, there may be developed a certain degree of tolerance. On the other hand, as shown in animals 16 and 17, there may be an increasing resistance to the hematin from the start as the toxic symptoms as well as the temperature decrease proportionally.

This subject of acquired tolerance has been taken up largely to emphasize the importance to be attached to results obtained from properly adjusted initial doses of hematin but also to explain the apparent discrepancies in the results from any series of hematin injections. It is the initial injection, with but few exceptions, that gives the maximum temperature reaction obtainable with a given dose of hematin until the series of injections has been extended to such a degree as to permit of the acquirement of a tolerance in highly susceptible animals or to cause a break in the early acquired tolerance of more resistant animals. When such conditions supervene, the temperature reaction may again increase and show an even greater response than with the initial injection (table I, animals 9 and 18).

SUMMARY.

The paroxysm of hematin intoxication in the rabbit undoubtedly presents many features of striking similarity to the paroxysm of human malaria; still one must hesitate to apply such results unreservedly in an attempt to identify the causative agent of the malarial paroxysm. When, in addition to the character of the paroxysm, we consider the sequence of events in the two instances, the analogy becomes so close that it seems impossible to regard the matter as a mere coincidence. The injection of hematin, especially in fractional doses, is in a measure comparable to the liberation of hematin into the human circulation by the malarial parasite. In these experi-

TABLE I.

Summary of Experimental Data.

No. of Animal: Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 1. Weight, 2.49.	Jan. 27, 1911	5.0			102.3	1.1		2	102.6	Rabbit hematin in all injections.
	Jan. 30, 1911		5.0	3.0	102.5		2.5	3	105.0	Highest temperature on secondary rise.
	Jan. 31, 1911		10.0	6.0	101.8		2.2	1½	102.8	
	Feb. 1, 1911		5.0	3.0	103.0		1.4	1½	103.0	
	Feb. 2, 1911		20.0	12.0	103.0		0.0		96.4	In two doses, 5 minutes apart. Died 3½ hours after injection.
No. 2. Weight, 2.78.	Feb. 1, 1911	10.0			102.8	1.8		2½	104.3	
	Feb. 2, 1911	10.0			102.2	1.8		1½	103.0	Rabbit hematin.
	Feb. 3, 1911	23.0			102.1	2.5		1	102.8	
	Feb. 7, 1911		5.0	2.6	102.9		3.1	1¼	106.0	Initial rise to 106° with fall to 105.3° F. and second rise.
	Feb. 8, 1911		2.5	1.3	102.6		1.2	1	103.0	
	Feb. 9, 1911		7.5	3.9	102.5		2.6	1¼	104.0	
	Feb. 11, 1911		7.5	3.9	102.4		3.0	1¼	103.9	
	Feb. 15, 1911		15.0	7.8	102.3		4.0	1¼	106.1	
	Feb. 16, 1911		20.0	10.4	102.8		3.3	1¼	104.9	
No. 3. Weight, 2.9.	Feb. 8, 1911		2.5		102.7					2.5 c.c. thick suspension of hematin in 0.85% salt solution. Died instantly.
No. 4. Weight, 2.65.	Feb. 8, 1911	10.0			102.6	1.4		1½	102.8	
	Feb. 9, 1911		7.5	4.2	102.2		2.6	1¼	104.6	Rabbit hematin.
	Feb. 11, 1911		7.5	4.2	102.3		2.8	1¼	103.8	
	Feb. 15, 1911		10.0	5.7	102.4		2.5	1¼	104.8	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 4. Weight, 2.65.	Feb. 16, 1911		15.0	8.4	101.6		2.1	3	103.7	Temperature dropped to 100.7° F., then rose very slowly.
No. 5.	May 1, 1911	7.0			101.8	1.3		1½	102.2	
Weight,	May 2, 1911		7.0	5.0	102.0		3.6	1½	105.0	Dog hematin.
2.85.	May 3, 1911		7.0	5.0	102.3		3.2	1¼	104.7	
No. 6.	May 1, 1911	8.0			102.4	1.6		1½	103.0	
Weight,	May 2, 1911	10.0			101.8	1.2		1½	102.2	Other manifestations very slight.
2.57.	May 3, 1911	15.0			102.0	1.7		1¼	103.3	
	May 4, 1911	20.0			102.1	2.1		1½	103.5	
No. 7.	May 1, 1911	8.0			101.6	1.5		2	102.0	
Weight,	May 2, 1911		8.0	4.7	101.2		3.7	1¼	104.0	Rabbit hematin.
2.53.	May 3, 1911		8.0	6.3	101.3		3.1	1¼	103.6	Dog hematin.
No. 8.	Oct. 18, 1911	6.0			102.1	1.4		1½	102.8	Hematin solution 5 months old, same as that used in in No. 7, injection 3.
Weight,	Oct. 20, 1911		6.0	10.0	102.3		1.8	1½	103.0	
1.85.										
No. 9.	Oct. 20, 1911	12.0			102.5	2.0		1½	102.7	
Weight,	Oct. 23, 1911		12.0	12.0	102.3		3.3	1	103.4	Ox hematin in all injections.
2.05.	Oct. 25, 1911		12.0	12.0	102.5		2.7	1¼	102.7	
	Oct. 26, 1911		12.0	12.0	103.6		2.0	¾	103.0	An afternoon experiment.
	Oct. 27, 1911		12.0	12.0	102.1		3.7	1	103.1	Temperature back to 103.6°
	Nov. 1, 1911		12.0	12.0	102.1		4.1	1¼	103.4	in 1½ hours.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 9. Weight, 2.05.	Nov. 3, 1911		12.0	12.0	101.8		4.3	1½	——	
	Nov. 6, 1911		18.0	18.0	101.5		4.9	1	103.5	Given in two doses 5 minutes apart.
No. 10. Weight, 1.85.	Oct. 25, 1911		12.0	20.0	102.6		0.8		103.0	Hematin solution 5½ months old, same as No. 7, injection 3, and No. 8, injection 2.
	Oct. 27, 1911		12.0	20.0	102.2		1.5		102.8	
No. 11. Weight, 2.0.	Oct. 27, 1911		5.0	7.5	102.6		0.6	1	102.5	Hematin solution, same as No. 10.
	Nov. 3, 1911	12.0			101.6	1.3		1½	101.6	Ox hematin in all other injections.
	Nov. 6, 1911	18.0			101.0	2.4		2	102.2	
	Nov. 8, 1911		6.0	6.0	101.4		2.6	1	102.8	
	Nov. 10, 1911		12.0	12.0	100.4		3.0	1¾	102.8	
	Nov. 13, 1911		18.0	18.0	100.3		3.9	3	104.2	
	Nov. 15, 1911		10.0	20.0	100.5		4.5	1½	103.0	
	Nov. 17, 1911		8.0	16.0	100.8		3.2	1¼	103.2	
No. 12. Weight, 2.0.	Nov. 10, 1911		15.0	15.0	101.2		3.2	1½	103.9	Ox hematin.
	Nov. 13, 1911		18.0	18.0	101.3		4.0	1½	105.0	
	Nov. 15, 1911		10.0	20.0	101.0		3.3	1	102.9	
	Nov. 17, 1911		8.0	16.0	101.7		3.1	1¾	104.0	
	Nov. 20, 1911		10.0	20.0	102.5		1.9	1½	103.3	In two doses 30 minutes apart. Initial rise checked by second dose.
	Nov. 22, 1911		5.0	10.0	101.4		3.4	1¼	103.4	Apparently about the optimum dose.
	Nov. 24, 1911		4.0	8.0	101.5		4.2	1¼	104.0	
	Nov. 27, 1911		8.0	16.0	101.4		3.0	1½	103.1	

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 13. Weight, 2.15.	Nov. 22, 1911	5.0			102.2	0.4		1	101.8	Ox hematin.
	Nov. 24, 1911		4.3	10.0	102.0		1.5	1	102.2	Single dose.
	Nov. 27, 1911		4.3	10.0	102.2		3.3	1¼	102.5	In 2 doses 30 minutes apart.
	Nov. 29, 1911	4.3			102.2	1.4		1¾	102.7	In 2 doses 30 minutes apart.
	Dec. 4, 1911	4.3			101.6	1.4		2½	102.8	In 2 doses 30 minutes apart.
	Dec. 6, 1911		4.3	10.0	101.8		3.0	1¾	103.6	In 2 doses 30 minutes apart.
	Dec. 8, 1911		2.1	5.0	101.7		2.1	1½	102.6	In 2 doses 30 minutes apart.
	Dec. 11, 1911		10.0	23.0	101.5		2.9	1¼	103.8	In 2 doses 30 minutes apart.
	Dec. 13, 1911		10.0	23.0	102.1		2.6	1	103.0	Single dose.
	Dec. 15, 1911		12.0	28.0	101.5		3.5	1	103.5	Single dose.
	Dec. 18, 1911		12.0	28.0	101.4		4.3	1¼	104.4	Given in 2 doses 30 minutes apart.
No. 14. Weight, 2.25.	Dec. 6, 1911	4.5			100.7	1.9		2¼	102.2	Ox hematin. Given in 2 doses 30 minutes apart.
	Dec. 8, 1911	2.2			100.4	1.6		2	——	Given in 2 doses 30 minutes apart.
	Dec. 11, 1911		2.2	5.0	101.0		3.2	1¾	103.4	Given in 2 doses 30 minutes apart.
	Dec. 13, 1911		3.3	7.5	101.4		3.4	1¾	103.6	Given in 3 doses 30 minutes apart.
	Dec. 15, 1911		4.5	10.0	101.5		2.6	1½	103.0	Given in 2 doses 30 minutes apart. Over-intoxication.
	Dec. 18, 1911		6.6	15.0	101.7		2.8	1½	104.5	Single dose. Over-intoxication.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours After Injection.	Remarks.
No. 15.	Jan. 12, 1912	3.2			100.6	1.5		1½	100.6	
Weight,	Jan. 16, 1912		3.2	10.0	99.8		3.2	1½	102.5	Ox hematin.
1.6.	Jan. 17, 1912		3.2	10.0	100.1		3.4	1½	102.3	
	Jan. 18, 1912		3.2	10.0	101.0		3.0	1	101.8	
	Jan. 19, 1912		3.2	10.0	101.2		2.5	1	102.6	
	Jan. 20, 1912		3.2	10.0	101.0		2.4	1½	101.9	
	Jan. 21, 1912									Another injection here without a record of temperature.
	Jan. 22, 1912		3.2	10.0	101.6		1.5	1½	102.0	
	Jan. 23, 1912		3.2	10.0	101.5		1.2	2½	102.7	Initial fall in temperature.
	Jan. 24, 1912		3.2	10.0	101.5		2.7	1	103.3	
	Jan. 25, 1912		3.2	10.0	101.8		3.2	1	104.0	
No. 16.	Jan. 12, 1912	3.3			101.1	2.7		1½	102.3	
Weight,	Jan. 16, 1912		3.3	10.0	100.6		3.9	1	104.4	Ox hematin.
1.65.	Jan. 17, 1912		3.3	10.0	101.8		2.8	1	102.9	
	Jan. 18, 1912		3.3	10.0	102.1		3.1	1	101.8	
	Jan. 19, 1912		3.3	10.0	102.0		2.9	1½	103.3	
	Jan. 20, 1912		3.3	10.0	102.5		3.2	1½	103.0	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		3.3	10.0	102.0		3.0	1½	103.1	
	Jan. 23, 1912		3.3	10.0	102.1		2.6	1½	103.0	
	Jan. 24, 1912		3.3	10.0	102.3		2.4	1	——	Leg broken by accident. Discontinued.

No. of Animal; Weight in Kilos.	Date of Injection.	Sodium Bicarbonate Salt Solution in c.c.	Hematin Solution in c.c.	Hematin per Kilo of Body Weight in mg.	Temperature (F.) at Time of Injection.	Elevation of Temperature with Sodium Bicarbonate Salt Solution.	Elevation of Temperature with Hematin.	Time in Hours to Fastigium.	Temperature 3 Hours after Injection.	Remarks.
No. 17.	Jan. 12, 1912	5.4			101.2	2.5		1½	103.2	Ox hematin.
Weight,	Jan. 16, 1912		5.4	15.0	101.3		4.0	3	105.3	Temperature reached 105° F. in 1 hour.
1.79.	Jan. 17, 1912		5.4	15.0	101.8		2.2	1	102.7	
	Jan. 18, 1912		5.4	15.0	101.5		3.6	1	102.6	
	Jan. 19, 1912		5.4	15.0	102.0		2.6	1½	103.3	
	Jan. 20, 1912		5.4	15.0	102.0		2.6	1½	103.3	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.4	15.0	102.2		2.8	1½	103.2	
	Jan. 23, 1912		5.4	15.0	102.1		2.9	1½	103.3	
	Jan. 24, 1912		5.4	15.0	102.6		2.8	1	103.3	
	Jan. 25, 1912		5.4	15.0	102.6		2.6	1	103.1	
No. 18.	Jan. 12, 1912	5.5			101.5	2.6		2	103.2	
Weight,	Jan. 16, 1912		5.5	15.0	101.6		3.4	3	105.0	Ox hematin.
1.84.	Jan. 17, 1912		5.5	15.0	102.0		2.8	1½	103.5	
	Jan. 18, 1912		5.5	15.0	101.9		2.5	1	103.6	
	Jan. 19, 1912		5.5	15.0	102.3		2.6	1½	103.8	
	Jan. 20, 1912		5.5	15.0	101.5		3.4	1½	103.7	
	Jan. 21, 1912									Injection here with no record.
	Jan. 22, 1912		5.5	15.0	101.9		3.8	1	103.4	
	Jan. 23, 1912		5.5	15.0	102.2		2.8	1½	104.7	
	Jan. 24, 1912		5.5	15.0	102.5		3.6	1	103.7	
	Jan. 25, 1912		5.5	15.0	102.7		3.3	1	104.6	

ments, both solution and finely divided suspensions of hematin have been found equally effective in eliciting the phenomena of the paroxysm, and while it seems possible that a portion of the malarial pigment might be dissolved in the alkaline human serum, such an assumption is probably not essential.

It might be objected that the toxic action of foreign hematin thus injected into the circulation would probably be greater than that of hematin derived from an animal's own blood, but as far as I have been able to determine, this objection does not seem valid, as rabbit hematin, dog hematin, and ox hematin produce in the rabbit effects that are alike in both character and degree.

The dose of hematin remains as the one factor to which it is possible to attach some degree of uncertainty, but even here the author feels that the range of experimental conditions has been kept within the bounds of legitimate analogy with conditions existing in the human subject of malarial infection.

Finally, the most conservative estimate of the value of such experiments points strongly to the fact that we have at least a potentially toxic substance in the pigment hematin as liberated by the malarial parasite into the circulation of the human host.

There is also abundant evidence to show that the action of hematin is not confined to the paroxysmal phenomena of malaria, but that other features of the disease may find their explanation in the action of this pigment. For the present, however, it seems advisable to confine the discussion to this one phase of the question.

CONCLUSIONS.

1. Alkaline hematin in doses commensurate with the amounts of hematin liberated in the human circulation by the segmentation of the malarial parasite, produces, when injected intravenously into the rabbit, a paroxysm which is characterized by a short prodromal stage, a stage of chill and rising temperature, and a hot stage. In their details the phases of this paroxysm are practically identical with the corresponding ones in the paroxysm of human malaria.

2. The phenomena in human beings infected with malaria are, at least in part, directly referable to the toxic action of this malarial pigment.

A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING.

First Paper: Splitting of the Complement Associated with Globulin Precipitation.*

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INTRODUCTION.

During the last few years much attention has been directed toward a phenomenon known as the splitting of complement. The conception is that complement, when treated according to certain procedures, can be decomposed into two definite portions each of which is by itself inactive but can act in combination with the other as complement. The two components of the complement differ in their behavior in hemolytic processes; one has the property of being bound by the sensitized erythrocytes, while the other possesses a lytic action upon sensitized cells that have been acted upon by the first component. Thus, according to certain investigators, complement is composed of an intermediary portion, called the mid-piece, and a lytic portion, called the end-piece. The terms "mid" and "end" are employed because the conception is that the mid-piece stands between the sensitized cells and the lytic portion of the complement, while the lytic portion when viewed graphically, becomes the end-piece.

In view of the high lability of complement it is natural to ask if the phenomenon of splitting brought about by certain procedures may not be due to some modifications of the complement, such as a reversible inactivation through changes in the reaction of the medium in which the complement is present. It appeared to us of interest to approach this question from a biochemical standpoint.

In our first paper we deal with the splitting phenomena which are

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associated with the precipitation of the globulin fraction, and in the second with those which occur without visible change in the physical conditions of the serum proteids.

HISTORICAL.

In fresh blood serum Landois found a hemolytic action upon alien corpuscles, and Fodor, Nuttall, and others, a bactericidal property, but none of the early investigators made exhaustive studies upon the nature of the active principles.

It was Hans Buchner who first pursued the study in a systematic manner and threw much light on this interesting phenomenon. Buchner (1890) established the fact that the active principle causing bacteriolysis or hemolysis is very labile and can be made inactive by a temperature of 55° C., by dialysis, or by dilution with distilled water. The active principle was designated by him alexin.

Bordet (1899) subsequently found that the alexin of Buchner is composed of two distinct principles, one a sensitizing substance and the other a thermolabile alexin. Somewhat later Ehrlich and Morgenroth (1899) made a similar observation, but by Ehrlich the sensitizing portion was called the amboceptor, and the alexin the complement. In this study Ehrlich and Morgenroth mention that the complement becomes inactive when mixed with water. Markl (1902) observed that sodium chlorid in concentration above 3 per cent. suppresses the activity of the complement.

While the disappearance of the complement activity after heating the serum to 55° C. was recognized by all as due to the destruction of the active principle at this temperature, no one was able to explain why the complement should lose its action in distilled water. This question was difficult to approach inasmuch as in a hypotonic salt solution a hemolytic experiment could not be carried out. Ferrata (1907), however, utilized the well known fact (noted by Hamburger, Madsen, and Arrhenius), that the corpuscles can be kept intact when suspended in an isotonic solution of sugar, and he studied the action of the salt-free complement (dialysed) in a salt-free isotonic medium, and found the complement to be inactive in this medium. Ferrata found also that during dialysis the serum separates into two portions, one the serum globulin (precipitate) and the other the albumin fraction (in solution). When tested separately in an isotonic salt solution neither of them caused hemolysis, while when united the action of complement was restored. He affirms that the component of complement remaining in the albumin portion is inactivated at 55° C.

The finding of Ferrata was confirmed by Brand, who showed further that the component carried down with the globulin fraction attaches itself to the sensitized corpuscles and that the component in the albumin fraction will afterwards act upon the cells so altered, He introduced, therefore, the terms mid-piece (Mittelstück) for the former and end-piece (Endstück) for the latter.

According to Brand the mid-piece of complement remains active when kept as precipitate (globulin) in distilled water, but loses its property on standing in salt solution. Brand and Tsurusaki (1908) found the mid-piece as well as the end-piece inactivated at 55° C.

Hecker (1907) and Sachs and Bolkowska state that the binding of the mid-

piece takes place at 0° C. as well as at higher temperatures, while that of the end-piece occurs only at higher temperatures. Thus they claim that the selective absorption of the natural hemolysin by means of corpuscles at 0° C. effects not only the fixation of the amboceptor, but also the mid-piece of complement.

Sachs and Teruuchi (1907) observed that the complement loses its activity in distilled water or isotonic sugar solution when the degree of dilution with distilled water is about 1 : 10, but not in much higher dilution. They were inclined to believe that the inactivation of the complement was caused by a certain ferment of the serum capable of exerting an injurious action only in a salt-free medium. They state that the inactivation of complement in a salt-free medium does not take place at a low temperature or in the presence of an excess of amboceptor. Complement a few days old does not undergo similar alteration (inactivation with water, etc.).

Tsurusaki found that the so-called splitting of complement is quite inconstant and seems to depend upon the quality of the dialysing membranes, upon the variations in different individuals of the same species of animal, and upon the temperature. For instance, the unaltered complement of rabbit was found in the supernatant fluid when dialysed at 0° C.

Sachs, and Sachs and Altmann (1908) then introduced a new method of splitting the complement. They used a weak solution of hydrochloric acid by which a certain portion of serum is precipitated (globulin). The precipitate corresponds with the mid-piece, and the supernatant fluid with the end-piece obtained by dialysis.

Later Michaelis and Skwirsky found that when corpuscles, amboceptor, and complement were put together in a medium containing a certain quantity of NaH_2PO_4 and Na_2HPO_4 in proportions resulting in isotonicity, there was no hemolysis. But on further analysis, they found that in this mixture the corpuscles had fixed not only the amboceptor, but also the mid-piece of complement. The corpuscles so modified were called persensitized, because they readily underwent hemolysis when the neutralized supernatant fluid (containing the end-piece) was added. In other words, persensitization can take place in a medium containing an excess of NaH_2PO_4 which suppresses the action of the end-piece, while the activity of the end-piece can be restored by neutralizing the excess of the acid phosphate with Na_2HPO_4.

Liefmann and Cohn introduced carbon dioxid for splitting the complement, whereupon the mid-piece is precipitated and the end-piece remains in solution. This phenomenon occurs only in a serum diluted with a sufficient quantity of water. They studied the influence of certain acids and alkalis and concluded that carbon dioxid exerts a far more inactivating effect than the acids or alkalis studied by them. Cholesterin inactivates the mid-piece in a salt-free medium. From the fact that the persensitization of corpuscles is affected with a minute amount of the mid-piece, they were inclined to think that this component acted like a ferment, an assumption held also by Rusznyák.

Jacoby and Schutze state that the complement inactivated by shaking and also the precipitate that is formed may be reactivated either by the mid-piece or the end-piece. On the other hand, the supernatant fluid of the shaken complement becomes active only with the end-piece.

Complement is known to be carried down by certain colloidal substances

through an adsorption (Landsteiner). Skwirsky confirmed this and points out a very interesting difference existing between the colloidal adsorption and the specific complement fixation as it occurs in the Bordet-Gengou or the Wassermann reaction. According to this author, later confirmed by Amako, in the former instance the end-piece as well as the mid-piece is carried down from the solution, while in the complement fixation the mid-piece alone is absorbed, leaving the end-piece free in the supernatant fluid.

Landsteiner, Liefmann and Cohn, and Fränkel state that the mid-piece prepared by the hydrochloric acid or carbon dioxid method from sera of different animals can persensitize the corpuscles for the end-piece of guinea pig serum, but not *vice versa*. This phenomenon is ascribed by them to the presence of natural amboceptor in the fraction containing the mid-piece. Fränkel points out certain differences in the behaviors of mid-pieces derived from the sera of different animals when these are acted upon by cholesterin, or sodium chlorid, or inactivated by the temperature of 57° C.

Marks observed that the reactivation of the end-piece by the mid-piece occurs when the latter is employed in a smaller quantity than the ratio of 1:1. He found that the heated as well as the fresh serum can furnish an active mid-piece, but a prolonged heating of the mid-piece at 55° C. finally destroys its function.

Liefmann studied the relation of certain lipoids and soaps to the mid- and end-pieces of complement and found that they have no effect upon the end-piece, but that they destroy the mid-piece. He brought out also the fact that persensitization can not take place unless the amount of amboceptor reaches nearly fifty units.

That the active principle of complement undergoes an inactive modification in the presence of various acids, alkalis, and salts has been carefully studied by Noguchi, von Liebermann, Hektoen, and others. According to the experiments of Noguchi, the complement is permanently injured by a strong solution of these substances, especially by the acids and alkalis, but when used within a certain limit, the activity can be restored with but slight loss of power.

EXPERIMENTAL PART.

There are at least five different procedures by which it is claimed that complement may be split[1] into two components. Sachs employs hydrochloric acid; Liefmann, carbon dioxid gas; Ferrata, dialyzes against distilled water; Michaelis and Skwirsky use a mixture of acid and alkaline phosphates; the fifth method is that of splitting by complement fixation.

[1] "Complement-splitting" is the term generally adopted by English speaking bacteriologists. This is the reason for the employment of the term in this paper, although a better word might be substituted, and the following work almost necessitates its replacement by the term "inactivation," since this is what has really been proved to take place in almost all cases of so-called splitting.

In our first paper we shall consider only the first three methods. In these the globulin fraction of the serum is precipitated during the process of splitting.

COMPLEMENT-SPLITTING WITH HYDROCHLORIC ACID.

The procedure introduced by Sachs is as follows: One cubic centimeter of guinea pig serum (complement) is mixed with 8.2 cubic centimeters of N/250 hydrochloric acid solution (in distilled water), and after standing at room temperature for one hour the mixture is centrifugalized, whereupon the precipitate settles down to the bottom leaving a clear supernatant fluid above. The supernatant fluid is carefully separated from the deposit[2] and is mixed with 0.8 of a cubic centimeter of N/25 sodium hydroxid solution (in 10 per cent. sodium chlorid). The addition of alkali is intended to neutralize the acid introduced into the serum for splitting, while the resultant solution is made isotonic at the same time (about 0.9 per cent. sodium chlorid). The deposit is washed with water and finally collected by centrifugalization, and then dissolved in 10 cubic centimeters of 0.9 per cent. sodium chlorid solution. The supernatant fluid and the precipitate, both now in clear solution, represent the end- and mid-piece, respectively. Thus, the supernatant fluid has no action upon the sensitized corpuscles unless the precipitate portion is added, and *vice versa*. Apparently the complement is separated into two components.

Experimental evidence was also brought forward to show that the end-piece or supernatant portion carries the labile zymotoxic component, and the mid-piece, or precipitate portion, possesses the property of enabling the end-piece to act upon corpuscles sensitized by amboceptor, and that it is not destroyed by the temperature of 56° C.

The above experiments have been confirmed by various workers, but we have been able to corroborate the results in part only, inasmuch as the supernatant fluid was found to be active upon sensitized corpuscles in most of our experiments (table I, experiment II) and was very seldom inactive.

[2] Finding that the supernatant fluid was often active without the addition of mid-piece, we took the precaution of filtering the end-piece through a hardened filter paper in order to be certain of the absence of the mid-piece.

TABLE I.

Complement-Splitting by Hydrochloric Acid.

Experiment I (Ideal Case).[3]

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.			Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10 per cent. NaCl.	Deposit dissolved in 10 c.c. of 0.9 per cent. NaCl.	Equal amounts of both ingredients.	After standing 1 hr. at room temperature (control).
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles and the total volume made up to 15 c.c. Results taken after 1 hr.			
1.0 c.c.	No H.[4]	No H.	——	
0.7 c.c.	No H.	No H.	——	
0.5 c.c.	No H.	No H.	C.H.	
0.4 c.c.			C.H.	
0.3 c.c.			C.H.	
0.2 c.c.			Sl.H.	C.H.
0.15 c.c.			Tr.H.	F.C.H.
0.1 c.c.			No H.	Tr.H.

Experiment II (Usual Splitting).

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.						Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.	
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10% NaCl.		Deposit dissolved in 10 c.c. of 0.9 % NaCl.		Equal volumes of both ingredients.		After standing at room temperature for 1 hr. (control).	
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles added to each tube and the total volume made up to 1.5 c.c. Results taken after 30 and 60 min.							
	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
1.0 c.c.	C.H.		No H.	No H.				
0.7 c.c.	C.H.		No H.	No H.	C.H.	C.H.		
0.5 c.c.	F.C.H.	C.H.			C.H.	C.H.		
0.4 c.c.	Mch.H.	C.H.			C.H.	C.H.		
0.3 c.c.	Sl.H.	C.H.			C.H.	C.H.		
0.2 c.c.	No H.	Sl.H.			F.C.H.	C.H.	C.H.	C.H.
0.15 c.c.		No H.			No H.	Sl.H.	C.H.	C.H.
0.1 c.c.						No H.	Sl.H.	Mch.H.
0.07 c.c.							No H.	No H.

[3] Throughout this work 0.9 per cent. sodium chlorid solution was used instead of 0.85 per cent. usually employed by other workers.

[4] In this and the following tables, for the sake of brevity, certain symbols are employed to indicate the degree of hemolysis; thus C.H. = complete hemolysis; F.C.H. = fairly complete hemolysis; Sl.H. = slight hemolysis; Tr.H. = trace of hemolysis; No H. = no hemolysis; Mch.H. = much hemolysis.

TABLE II.

The Effect upon Components of Guinea Pig Serum of Heating for 30 Minutes at 56° C.

	Experiment I.[5]			*Experiment II.*			
	Fresh guinea pig serum 1 c.c.+8.2 c.c. HCl N/250 (in distilled water).			Guinea pig serum heated at 56° C. for 30 minutes + 8.2 c.c. HCl N/250 (in distilled water).			
	Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.			Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.			
	0.15 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.			0.2 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.			
	Results taken after:			Results taken after:			
	30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	0 control.
+0	No H.	Sl.H.	C.H.	No H.	No H.	No H.	
+Alanin 0.3 c.c. of 7.5% solution in 0.9% NaCl.	C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
+Deposit from splitting in experiment I dissolved in 10 c.c. 0.9% salt solution. 0.15 c.c.	C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
0.07 c.c.	C. H.	C.H.	C.H.	No H.	No H.	No H.	
+Deposit from splitting in experiment II dissolved in 10 c.c. 0.9% salt solution. 0.2 c.c.	No H.	Sl.H.	C.H.	No H.	No H.	No H.	No H.
0.1 c.c.	C.H.[6]	C.H.	C.H.	No H.	No. H	No H.	

While the phenomenon suggests a double constitution of the complement, yet it does not exclude the possibility of its being caused by an excess of acid or alkali. Guinea pig serum, when mixed with hydrochloric acid in the proportion prescribed in this procedure, becomes almost neutral or but faintly alkaline. This causes the precipitation of certain proteids, mainly the globulins. Thus the acid introduced is quickly taken up by certain alkaline salts of the serum and there is none left free. The precipitated globulins also bind a certain amount of the acid. The supernatant fluid which is almost neutral and contains chiefly the albumin fraction of the serum, is then mixed with sufficient free alkali to neutralize exactly

[5] The total volume was brought up to 1 c.c. by 0.9 per cent. salt solution in both experiments.

[6] This phenomenon does not occur constantly. With some specimens of complement (very few) the mid-piece is undoubtedly destroyed by heating the whole serum previous to the splitting, but it is easier to destroy the mid-piece after its separation.

the amount of acid added, but this amount of alkali is in excess when added to the serum mixture from which the globulin fraction was removed after its precipitation by hydrochloric acid. Thus it happens that in the serum treated with the acid and then neutralized with the alkali in the manner prescribed, in the presence of the globulin fraction, the complement action is restored. On the other hand, if the globulin fraction is first removed and the same amount of the alkali is added to the remaining supernatant fluid, the complement activity of the supernatant fluid is paralyzed. The exami-

TABLE III.

Demonstration of Complement Inactivation by Sodium Hydroxid in the Process of Complement-Splitting.

	Tubes.					
	1	2	3	4	5	6
Guinea pig serum.....	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.
N/250 HCl.....	8.2 c.c.	8.2 c.c.	8.2 c.c.	8.2 c.c.	———	———
0.9% NaCl.....					9.0 c.c.	
Distilled water.....						8.2 c.c.

After 1 hour at room temperature all the tubes were centrifugalized. From tubes 1 and 2 only supernatant fluid was used; from the others the entire contents of the tubes were used.

N/25 NaOH in 10% NaCl.....	0.8 c.c.	———	———	0.8 c.c.	———	———
10% NaCl.....	———	0.8 c.c.	0.8 c.c.	———	———	0.8 c.c.
	Supernatant fluid		The entire contents of the tubes			
Reaction of resulting fluids to alizarin-indigo[7] (calculated for the whole volume).	5.6 c.c. HCl N/250	2.5 c.c. NaOH N/250	2.5 c.c. NaOH N/250	5.6 c.c. HCl N/250	6.2 c.c. HCl N/250	6.2 c.c. HCl N/250
Complement activity of the resulting mixtures (with 0.1 c.c. of 10% suspension of corpuscles and 2 units of amboceptor. Amounts of mixture. 1.0 c.c.	C.H.					No H.
0.7 c.c.	Sl.H.					
0.5 c.c.	Tr.H.			C.H.		
0.3 c.c.	No H.	C.H.	C.H.	F.C.H.		
0.2 c.c.		F.C.H.	F.C.H.	Mch.H.	C.H.	
0.15 c.c.		Mch.H.	Sl.H.	Tr.H.	F.C.H.	
0.1 c.c.		No H.	No H.	No H.	Sl.H.	
0.07 c.c.					No H.	

[7] The use of this indicator, first introduced by Bernhardt, was suggested to us by Prof. W. J. Gies. The indicator is not noticeably influenced by the presence of proteids or carbon dioxid.

nation of the so-called neutralized supernatant fluid for alkalinity indicates a slight excess of alkali over that present in the same dilution of the untreated serum.

That this slight excess of alkalinity is not solely responsible for the loss of action of the untreated complement, but that the nature of the alkali employed has more to do in paralyzing the complement seems very probable. Thus, the free alkali in the form of sodium hydroxid fails to restore the original alkaline salts, and exerts an injurious effect upon the active complement constituents. That this is the case is easily shown by the experiments in table III.

These experiments show that the supernatant fluid from which the native alkalinity has been almost completely removed by hydrochloric acid still has nearly two thirds of the original complement power of the serum. But by adding gradually increasing amounts of sodium hydroxid, the activity is steadily reduced (table V) until in some cases it disappears completely (table VI), when the amount of sodium hydroxid reaches the point supposed to be necessary to restore the original alkalinity of the treated serum. The completeness of the inactivation of the complement action of the supernatant fluid is also enhanced by a slight excess of the free sodium hydroxid toward which the complement shows a great susceptibility. It may be assumed that the salts that cause the native alkalinity, principally the carbonates and phosphates, are split by hydrochloric acid and form sodium chlorid and the acids, but the greater part of carbon dioxid leaves the fluid, thus causing the free alkali introduced to act directly upon the complement.

It is interesting, however, that, when the serum was acted on by hydrochloric acid and sodium hydroxid was subsequently added, without removing the globulin fraction, much if not all of the complement activity was restored (table IV, A).

The reason for sodium hydroxid not causing the same injurious effect upon the complement in this instance (table IV, A) must be due to the presence of the globulin fraction which seems to have a greater affinity for the free sodium hydroxid than the albumin fraction in which the active principle of the complement seems to reside.

That the globulin fraction has a greater affinity for sodium

TABLE IV.

Comparison of Complement Inactivation by Different Alkalis in the Presence and Absence of Globulin Fractions.

Amounts of neutralized dilution used.	To 1 c.c. of guinea pig complement 8.2 c.c. of N/250 HCl were added and kept at room temperature for 1 hr.								1 c.c. complement + 8.2 c.c. HCl N/250 in 0.9% NaCl.		1 c.c. complement + 9 c.c. 0.9% NaCl.	1 c.c. complement + 8.2 c.c. distilled water.
	After 1 hr. corresponding amounts of alkali were added to neutralize the acidity and made isotonic.						Made isotonic without neutralization (control).		Neutralized with NaOH.	Non-neutralized.		Made isotonic.
	NaOH		$NaHCO_3$		Na_2HPO_4							
	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Whole serum.		
1.0 c.c.	C.H. No H.	C.H. Sl.H. No H.	C.H. F.C.H. Sl.H. No H.	C.H. Mch.H. Sl.H. No H.	C.H. Sl.H. No H.	C.H. Sl.H. No H.	C.H. F.C.H. No H.	C.H. F.C.H. No H.	Mch.H. Sl.H. No H.	C.H. Mch.H. Sl.H. No H.	C.H. Sl.H. No H.	No H.
0.7 c.c.												
0.5 c.c.												
0.3 c.c.												
0.2 c.c.												
0.15 c.c.												
0.1 c.c.												
0.07 c.c.												
	A		B		C		D		E		F	G

TABLE V.

The Effect of the Excess of Hydrochloric Acid and Sodium Hydroxid upon the Serum from Which the Globulin Fraction Is Removed.

1 c.c. of complement + 8.2 c.c. of N/250 HCl at room temperature for 1 hour, then centrifugalized and the globulin fraction removed. Only the supernatant fluid was used after it was made isotonic by 10 per cent. NaCl.

Amounts of mixtures.	1 c.c. of supernatant fluid in all tubes, then different amounts of HCl or NaOH were added and the resultant mixtures tested in varying amounts.									Native complement + 1 c.c. 0.9% NaCl 9 c.c.
	N/250 HCl				Original isotonized supernatant fluid. 0 (non-neutralized.)	N/250 NaOH.				
	0.8 c.c.	0.6 c.c.	0.4 c.c.	0.2 c.c.		0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	
0.5 c.c.	No H	Mch.H.							Sl.H.(?)	
0.3 c.c.	No H.	F.C.H.	C.H.				C.H.	C.H.	C.H.	
0.2 c.c.	Sl.H.	C.H.	F.C.H.(?)	C.H.			F.C.H.	F.C.H.	F.C.H.	
0.1 c.c.	Sl.H.	C.H.	F.C.H.(?)	F.C.H.(?)			F.C.H.	F.C.H.	F.C.H.	
0.07 c.c.	Tr.H.	Sl.H.	F.C.H.(?)	F.C.H.(?)	C.H.	C.H.	F.C.H.	Sl.H.	Sl.H.	
0.05 c.c.	Tr.H.(?)	Tr.H.	Mch.H.	F.C.H.(?)	F.C.H.(?)	F.C.H.	Sl.H.	Sl.H.	Sl.H.	
0.03 c.c.	No H.	No H.[1]	Sl.H.	Sl.H.	F.C.H.(?)	Sl.H.	Sl.H.	Sl.H.	No H.[8]	
0.02 c.c.			No H.	No H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	No H.	C.H.
0.01 c.c.					No H.	No H.	Tr.H.(?)	Sl.H.	No H.	F.C.H.
0.005 c.c.							No H.	No H.		No H.

[8] So-called "Endstück."

hydroxid than the albumin fraction is easily demonstrated by experiments described later.

In order to ascertain whether the loss of complement action in the albumin fraction, the so-called end-piece, is really due to a reversible inactivation through the action of sodium hydroxid, such as had been observed by Noguchi in his earlier experiments, and not due to the removal of one of the interdependent components, the mid-piece, it was necessary to make a series of reverse experiments with an acid.

The following experiments show that the complement activity of the inactive end-piece can be restored to a great extent by means of neutralization of the alkali with adequate amounts of hydrochloric acid (table VII).

We now come to consider the mechanism of the restoration of the complement activity of the end-piece by the globulin fraction known as the mid-piece. From the reactivation experiments with hydrochloric acid, it is not improbable that the combination of the complement and sodium hydroxid can also be split by means of an amphoteric compound. Among the amphoteric substances we find various proteids, proteoses, and many non-coagulable synthetic amino acids. The serum globulins are undoubtedly amphoteric.

In the present experiments we have also used egg-white, alanin, deuteroalbumose, and peptone.[9]

These experiments (table VII) demonstrate that the complement action of the so-called end-piece can be readily restored by adding adequate quantities of the amphoteric substances herein employed. One may wonder how such a weak acid as the amino acid, which does not form a salt at ordinary temperature, can reverse the combination of the alkali and the complement fraction. But, it must be remembered that the affinity between the complement and alkali cannot be stronger than that which exists between any other amphoteric bodies. Moreover, the direction of the reversion is dependent upon the total number of acid radicals possessed by a given substance. Thus, a greater amount of the substance is

[9] We are indebted to Dr. F. J. Birchard, of The Rockefeller Institute for Medical Research, for the albumose and peptone preparations. The albumose was prepared from fibrin according to Pick's method, and the peptone from Witte's peptone according to Sigfried's method.

TABLE VI.

Comparative Action of Sodium Hydroxid upon the Whole Complement and upon the Albumin Fraction Alone.

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Supernatant fluid (after centrifugalization) of the mixture. 1 c.c. guinea pig serum + 8.2 c.c. HCl N/250 for 1 hour at room temperature.							
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3			C.H.	F.C.H.	Sl.H.	Sl.H.	No H.	
		Alanin 0.3					C.H.	C.H.	Mch.H.	No H.
	0.01	Salt 0.3					C.H.	Sl.H.	No H.	
		Alanin 0.3						C.H.	No H.	
	0.015	Salt 0.3				C.H.	Mch.H.	No H		
		Alanin 0.3					C.H.	F.C.H.	F.C.H.	No H.
	0.02	Salt 0.3				C.H.	F.C.H.	Tr.H.	No H.	
		Alanin 0.3					C.H.	Tr.H.	No H.	
	0.03	Salt 0.3				C.H.	Tr.H.	No H.		
		Alanin 0.3				C.H.	F.C.H.	No H.		
	0.04	Salt 0.3	C.H.	F.C.H.	No. H.					
		Alanin 0.3			C.H.	F.C.H.	F.C.H.	No H.		
	0.05	Salt 0.3	C.H.	F.C.H.	No H.					
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		
	0.07[9]	Salt 0.3	No H.							
		Alanin 0.3				C.H.	Tr.H.	No H.		
	0.1	Salt 0.3	No H.							
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Guinea pig complement 1 c.c. + 9 c.c. 0.9% NaCl for 1 hour at room temperature (control).								
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.	0.05 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3							C.H.	F.C.H.	F.C.H.
		Alanin 0.3									C.H.
	0.01	Salt 0.3									C.H.
		Alanin 0.3									F.C.H.
	0.015	Salt 0.3									C.H.
		Alanin 0.3								F.C.H.	Sl.H.
	0.02	Salt 0.3								C.H.	C.H.
		Alanin 0.3							Sl.H.	No H.	
	0.03	Salt 0.3							C.H.	Tr.H.	No H.
		Alanin 0.3				C.H.	F.C.H.	Mch.H.	Sl.H.	No H.	
	0.04	Salt 0.3					C.H.	Mch.H.	Sl.H.	No H.	
		Alanin 0.3			C.H.	F.C.H.	No H.				
	0.05	Salt 0.3				C.H.	F.C.H.	No H.			
		Alanin 0.3									
	0.07[10]	Salt 0.3	C.H.	Sl.H.	No H.						
		Alanin 0.3			C.H.	F.C.H.	No H.				
	0.1	Salt 0.3	No. H.								
		Alanin 0.3			C.H.	F.C.H.	Tr.H.(?)	No H.			

In this experiment sheep corpuscles were used (0.5 c.c. of 5 per cent. suspension) and 50 units of amboceptor.

[10] The amount of sodium hydroxid used in the original complement-splitting experiments corresponds to 0.08 c.c. in this series.

TABLE VII.

Reactivation of the End-Piece of the Complement Split by Hydrochloric Acid.

	Alanin (5%), 0.075 c.c.	α peptone (2%), 0.1 c.c.	Deutero-albumose B (2%), 0.05 c.c.	Mid-piece from HCl-splitting, 0.05 c.c.	Mid-piece + NaOH N/250 (1 c.c. + 0.25 c.c.), 0.05 c.c.	Mid-piece heated 30 min. at 56° C., 0.05 c.c.	Sheep serum diluted 1:10 heated 56° C., 0.05 c.c.	Mid-piece from dialysis, 0.05 c.c.	Mid-piece from dialysis + HCl ℥ (3:1), 0.05 c.c.	HCl N/250	0 control.
Native complement 0.03 c.c. inactivated by 0.6 c.c. NaOH N/250	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	0.005 c.c. C.H.	No H.
End-piece[11] non-neutralized, 0.02 c.c.	No H.	No H.	Sl.H.	Sl.H.	F.C.H.	F.C.H.	Mch.H.	Mch.H.	Tr.H.	0.005 c.c. No H.	Tr.H.(?)
End-piece[12] 9.2 c.c. + 0.2 NaOH N/25 in NaCl, 0.05 c.c.	Sl.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Tr.H.(?) Sl.H.(?)
End-piece[12] 9.2 c.c. + 0.4 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	F.C.H.	F.C.H.	Sl.H.	Tr.H.	F.C.H.	F.C.H.	0.02 c.c. F.C.H.	
End-piece[12] 9.2 c.c. + 0.8 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	Sl.H.	Mch.H.	F.C.H.	Tr.H.	Sl.H.	Sl.H.	0.04 c.c. C.H.	Tr.H.
End-piece[12] 9.2 c.c. + 0.4 c.c. HCl N/25, 0.03 c.c.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Mch.H.	Tr.H.	Tr.H.	0.005 c.c. Tr.H.	Tr.H.
Control 0	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	

[11] The end-piece resulted from mixing 1 c.c. of complement + 8.2 c.c. N/250 HCl at room temperature for 60 minutes. Then the mixture was centrifugalized and the supernatant fluid made isotonic and diluted to 10 c.c.

[12] All the mixtures, beginning with the third end-piece, before being made isotonic were neutralized (partly) by the amounts of NaOH shown, then made isotonic and, if necessary, diluted to 10 c.c.

required if it possesses fewer acid radicals. That the globulins have a greater affinity for sodium hydroxid than the albumins may be accounted for by the larger size of the molecules of the former class of proteids. The solubility of the substances seems also to be important, as certain insoluble amino acids, such as asparagin, asparagin acid, and leucin failed to reactivate the end-piece.

COMPLEMENT-SPLITTING WITH CARBON DIOXID.

Liefmann splits the complement in the following manner. One half of a cubic centimeter of guinea pig serum (complement) is mixed with two cubic centimeters of distilled water, and carbon dioxid gas is passed through until no more precipitate is formed. The treated serum is then centrifugalized to separate the precipitate from the supernatant fluid. The clear supernatant fluid is carefully decanted and filtered through hardened filter paper into another receptacle and is used as the end-piece after being made isotonic with sodium chlorid. The deposit, which represents the globulin fraction of the serum, is then dissolved in 2.5 cubic centimeters of 0.85 per cent. salt solution. This portion is used as the mid-piece. In a certain number of instances the phenomenon of splitting is ideally shown, the end- and mid-pieces separately having no action upon the sensitized corpuscles (table X). The two components obtained by the carbon dioxid method behave in much the same way as those prepared by the hydrochloric acid method of Sachs. It may be stated, however, that the end-piece portion remains quite active in many instances, as has been observed also by Liefmann and others (tables IX and X).

In considering the processes that lead to the inactivation of the end-piece by carbon dioxid and the subsequent reactivation of this portion by the mid-piece of the globulin fraction, one can conceive that an essential difference exists between the inactivation by carbon dioxid and that by the hydrochloric acid method. In case of the hydrochloric acid-splitting, the carbonates as well as other alkaline salts of the serum are completely converted into chlorids. Small amounts of free carbon dioxid or other weak acids remaining in the fluid cause a partial inactivation of the complement.

On the other hand, the passage of carbon dioxid through a

TABLE VIII.

Inactivation of Complement by Carbon Dioxid.

Dilution 1:10 in water.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.04 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.005 c.c.
Native complement in NaCl									C.H.	F.C.H.	No H.
Complement + CO_2, 2 min. } the whole						C.H.	Tr.H.	No H.			
Complement + CO_2, 10 min. } made isotonic				C.H.	F.C.H.	No H.	No H.				
Supernatant fluid alone											
Guinea pig complement after passage of CO_2, 2 min.				C.H.	Sl.H.	No H.					
Guinea pig complement after passage of CO_2, 4 min.			C.H.	F.C.H.	F.C.H.	Tr.H.(?)	No H.				
Guinea pig complement after passage of CO_2, 6 min.			C.H.	Tr.H.	No H.						
Guinea pig complement after passage of CO_2, 8 min.		C.H.	Mch.H.	No H.							
Guinea pig complement after passage of CO_2, 10 min.	C.H.	F.C.H.	Mch.H.	No H.							

TABLE IX.

Splitting by Carbon Dioxid.

	Amount of end and mid-piece.							
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10				C.H.	F.C.H.	Mch.H.	Tr.H.	No H.
1:10 complement (CO_2), 10 min. (whole)				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction, CO_2, 10 min. (end-piece)	C.H.	F.C.H.	Mch.H.	Tr.H.(?)	No H.			
End-piece[13] (CO_2), 10 min. +mid-piece, 10 min. 1:1			C.H.	F.C.H.	F.C.H.	Tr.H.	No H.	
End-piece (CO_2), 10 min. +mid-piece, 60 min. 1:1,		C.H.	F.C.H.	Mch.H.	No H.			
Albumin fraction (CO_2), 60 min. (end-piece)			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
End-piece, 60 min., +mid-piece, 10 min., 1:1	F.C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.(?)	No H.		
End-piece, 60 min., +mid-piece, 60 min., 1:1			C.H.	F.C.H.	Mch.H.	No H.		
Mid-piece, 10 min., alone	No H.							
Mid-piece, 60 min., alone	No H.							

[13] 1:1 means equal amounts of both end- and mid-pieces; therefore in the column under 0.2 c.c. it means 0.2 c.c. of end-piece +0.2 c.c. of mid-piece.

TABLE X.

A Case of Successful Splitting by Carbon Dioxid (Very Rare).

	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.
Native complement 1:10 dilution							C.H.	Sl.H.	No H.	
Complement + CO_2, 10 min., made isotonic (whole)							C.H. (delay)	Sl.H.	No H.	
Albumin fractions alone	No H.									
Albumin fraction + globulin fraction 1:5					C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
Globulin fraction alone	No H.									

A Case of Imperfect Splitting by Carbon Dioxid.

	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.
Native complement 1:10 dilution				C.H.	F.C.H.(?)	F.C.H.	Sl.H.	No H.
Complement + CO_2, 10 min., made isotonic (whole)				C.H.	F.C.H.	Mch.H.	No H.	
Albumin fraction alone				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction + globulin fraction 1:5	C.H.	F.C.H.	Sl.H.	Sl.H.	No H.			

A Case of the Complement Twenty-four Hours Old.

	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.
Native complement 1:10 dilution	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	
Complement CO_2, 10 min., made isotonic (whole)		C.H.	F.C.H.	Mch.H.	Tr.H.	No H.	
Albumin fraction alone			C.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Albumin fraction + globulin fraction 1:1	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	

TABLE XI.

Inactivation of Complement by Carbon Dioxid in Different Media.

	+ CO_2, 10 min.						
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.
Native complement 1:10 in NaCl							
Complement 1 c.c. +8.2 c.c. distilled water, isotonized after 30 min.			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. +8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	Sl.H.	No H.
Complement 1 c.c. +8.2 c.c. N/250 HCl (in distilled water), neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. +8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.				C.H.	Mch.H.	Sl.H.	Tr.H.
Complement[14] in isotonic sugar solution 1:10+0.3 c.c. NaCl 0.9%	C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	

	No CO_2.							
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10 in NaCl						C.H.	F.C.H.	No H.
Complement 1 c.c. +8.2 c.c. distilled water, isotonized after 30 min.	Tr.H.	No H.						
Complement 1 c.c. +8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	C.H.	F.C.H.	No H.
Complement 1 c.c. +8.2 c.c. N/250 HCl (in distilled water) neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	
Complement 1 c.c. +8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.		C.H.	C.H.	C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement[14] in isotonic sugar solution 1:10+0.3 c.c. NaCl 0.9%						C.H.	Sl.H.	No H.

[14] The addition of sodium chlorid is to prevent complement from complete inactivation.

TABLE XII.

Reactivation of Complement Inactivated by Carbon Dioxid.

Amounts of reactivating substances.	Alanin 3 %.	Mid-piece (CO_2).	Egg-white 1:10.	Sheep serum, 30 min. at 56° C. 1:5.	Protoalbumoses 5 %.	Deuteroalbumoses 2 %.
0.3 c.c. of end-piece from CO_2-splitting in each tube. + 1.0 c.c.						
+ 0.7 c.c.						
+ 0.5 c.c.						
+ 0.4 c.c.	C.H.					
+ 0.3 c.c.	C.H.	Sl.H.				
+ 0.2 c.c.	C.H.	Mch.H.				
+ 0.15 c.c.	F.C.H.	Mch.H.			No. H.	
+ 0.1 c.c.	Mch.H.	C.H.		Sl.H.	Sl.H.	No. H.
+ 0.05 c.c.		Mch.H.		Sl.H.	Mch.H.	Tr.H.
+ 0.03 c.c.		Sl.H.	C.H.	F.C.H.	Sl.H.	Mch.H.
+ 0.02 c.c.			C.H.			Sl.H.
+ 0.01 c.c.			C.H.			
+ 0.005 c.c.			C.H.			
+ 0						

mixture of guinea pig serum and water precipitates the globulin fraction just as in the case of the hydrochloric acid treatment, but the carbonates as well as phosphates remain unaffected by this treatment. The inactivation of the complement in this instance is doubtless due to the overcharging of the serum water with carbon dioxid gas, which by virtue of its acid nature combines with the amphoteric molecules of the serum albumin fraction in which the activity of the complement is residing. In a medium, whether in the undiluted native serum or in a serum diluted with sodium chlorid solution of isotonic concentration, in which the sodium chlorid content reaches a sufficient concentration (table XI), a weak acid like carbon dioxid fails to enter into even a loose combination with the albumin molecules, and hence leaves the complement comparatively uninjured. The reason that carbon dioxid acts upon the complement molecules in serum sufficiently diluted with water is that the strong electrolyte, sodium chlorid, can in that dilution no longer interfere with the action of a weak acid. In diluted serum carbon dioxid causes an acid inactivation of complement and the activity of complement may be restored by certain amphoteric substances (table XII) and also by an alkali (table XV, C), but not by an acid.

The phenomenon of reactivation by means of adequate quantities of sodium hydroxid may raise a question as to why the result-

TABLE XIII.

Substances Subjected to Carbon Dioxid Lose Their Reactivating Power for Complement Inactivated by Carbon Dioxid.

Substances acted upon by carbon dioxide for 15 minutes. The total volume in each tube was 1.5 c.c.

	Alanin 5%.				Mid-piece (CO_2).				o Control.
	No CO_2.		+ CO_2		No CO_2.		+ CO_2.		
	0.15 c.c.	0.1 c.c.	0.15 c.c.	0.1 c.c.	0.07 c.c.	0.03 c.c.	0.07 c.c.	0.03 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	No H.	Tr.H.	C.H.	C.H.	Tr.H.	Sl.H.(?)	No H.
No end-piece (control)	No H.		No H.		No H.		No H.		

	Egg-white (1:10).				Sheep serum 56° C. (1:5).				o Control.
	No CO_2.		+ CO_2.		No CO_2.		+ CO_2.		
	0.01 c.c.	0.005 c.c.	0.01 c.c.	0.005 c.c.	0.03 c.c.	0.01 c.c.	0.03 c.c.	0.01 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	Tr.H.	Tr.H.	C.H.	F.C.H.	Tr.H.	Mch.H.	No H.
No end-piece (control)	No H.		No H.		No H.		No H.		

ant Na_2CO_3 or $NaHCO_3$ no longer exerts the same effect as the free sodium hydroxid and carbon dioxid, since these are readily dissociable in the fluid. But one must not forget that in the stage of reactivation the fluid is now made isotonic with sodium chlorid, and this restrains the dissociation of carbonates or phosphates sufficiently to prevent the free action of the carbon dioxid or sodium ions upon the complement fraction. It is important to notice that an acid has no reactivating property for the end-piece prepared with carbon dioxid, while exactly the opposite is the case with the end-piece made with hydrochloric acid, which is an inactivation by alkali.

In the experiments in table XIII we show that amphoteric substances capable of reactivating complement inactivated by carbon dioxid lose this reactivating property if treated with carbon dioxid before being added to the inactive complement. The way in which amphoteric substances reactivate complement is thus clearly demonstrated.

COMPLEMENT-SPLITTING BY MEANS OF DIALYSIS.

Before Sachs, Liefmann, and others introduced various methods of splitting the complement, Ferrata discovered that by dialyzing complement against water through a fish membrane or a celloidin sac, it could be separated into two inactive components, an albumin fraction corresponding with the end-piece of the later investigators, and a globulin fraction (which precipitates during dialysis) corresponding with the mid-piece. This result, although not always obtained, was nevertheless confirmed by other workers.

On several occasions in the present series of experiments we have also confirmed this finding. Our explanation for this phenomenon is, however, entirely different from that given by previous workers. As will be shown in the following protocols, the supernatant fluid (albumin fraction) when separated from the precipitate (globulin fraction) and made isotonic with sodium chlorid, is quite inactive upon the sensitized corpuscles, but can be reactivated by adding, not only the globulin fraction of the same serum (isotonized with sodium chlorid), but also the globulin fraction prepared by the hydrochloric acid or carbon dioxid methods (table XIV). Indeed,

TABLE XIV.

Reactivation of Complement Inactivated by Distilled Water or Dialysis.[15]

	Alanin 5%.			Mid-piece (CO_2, 10 min.).			Mid-piece dialysis.		
	0.3 c.c.	0.15 c.c.	0.07 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
Complement+distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl).....0.2 c.c.	C.H.	Sl.H.	No H.	Mch.H.	C.H.	F.C.H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10).....0.2 c.c.	C.H.	F.C.H.	No H.	F.C.H.	C.H.	F.C.H.	No H.	No H.	Sl.H.
End-piece (CO_2) (final dilution 1:10) (control).....0.2 c.c.	C.H.	F.C.H.	No H.	Sl.H.	F.C.H.	C.H.	Sl.H.	F.C.H.	No H.
No complement. Control.....	No H.			No H.			No H.		

	Egg-white (1:10).			Sheep serum (56° C.) 1:5.			Control. No mid-piece.
	0.1 c.c.	0.05 c.c.	0.03 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	
Complement+distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl).....0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10).....0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
End-piece (CO_2) (final dilution 1:10) (control).....0.2 c.c.	Tr.H.	Sl.H.	C.H.	C.H.	C.H.	C.H.	No H.
No complement. Control.....	No H.			No H.			

[15] Results taken after incubation at 37° C. for 1 hour.

the reactivation may be even better and more constant with mid-pieces prepared by these other methods than with its own mid-piece. Further, it is found that an acid (table XV, B) or certain amphoteric substances which bear no relation to the serum (table XIV)

TABLE XV.

Reactivation of Complement Inactivated by Acid (Carbon Dioxid) Compared to Reactivation of Complement by Alkali (Dialysis or Distilled Water).

A

Incubation time.		Complement + distilled water (1:10) at room temperature for 1 hr.; the whole made isotonic; 0.5 c.c. used in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
HCl N/250	0.1		No H.	No. H.	Tr.H.	No. H.	Sl.H.
	0.125		No H.	Tr.H.	Sl.H.	Sl.H.	F.C.H.
	0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
Control		F.C.H.	F.C.H.	C.H.	C.H.	No H.	No H.(?)
NaOH N/250	0.1						
	0.2						
	0.3	No H.	No H.	No H.	No. H.		
	0.4	No H.	Tr.H.(?)	No H.	Tr.H.	No H.	No H.
	0.5	Tr.H.	Tr.H.	Sl.H.(?)	Sl.H.	No H.	No H.

B

Incubation time.		End-piece of complement dialyzed for 12 hrs. against running water; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
HCl N/250	0.1		No H.	No H.	No H.	No H.	No H.
	0.125		No H.	Sl.H.	Mch.H.	Sl.H.	Mch.H.
	0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
Control		F.C.H.	C.H.	C.H.	C.H.	No H.	Tr.H.
NaOH N/250	0.1						
	0.2						
	0.3		No H.		No H.		No H.
	0.4		No H.		No H.		No H.
	0.5		Tr.H.		Tr.H.		No H.

	Incubation time.	End-piece (CO_2) for 10 min.; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
		+ Alanin 7.5%.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
C	HCl N/250 0.1		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.125		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.15		No H.		No H.	No H.	Tr.H.
	Control	Sl.H.	C.H.	Tr.H.	C.H.	No H.	Sl.H.
	NaOH N/250 0.1	No H.	Tr.H.	No H.	Tr.H.		
	NaOH N/250 0.2	Tr.H.	F.C.H.	Tr.H.	C.H.	C.H.	
	NaOH N/250 0.3	Mch.H.	C.H.	Mch.H.	C.H.	C.H.	
	NaOH N/250 0.4						
	NaOH N/250 0.5						

reactivate this dialyzed end-piece. On the other hand, alkalis do not reactivate it (table XV, B).

From the experiments given above it becomes highly probable that dialyzed serum loses its complement activity chiefly by an alkali inactivation of a reversible character. The cause of this

TABLE XVI.

Rate of Dialysis of Sodium Chlorid and $NaHCO_3$ from the Mixture of Fifty Cubic Centimeters of 1 per cent. Sodium Chlorid + Fifty Cubic Centimeters N/50 $NaHCO_3$.

	NaCl				$NaHCO_3$			
	$AgNO_3$	NH_4SCN	Combined Ag	NaCl	H_2SO_4 N/100	NaOH N/100	Combined acid	$NaHCO_3$ N/50
After[16]								
0	4.0 c.c.	2.0 c.c.	2.0 c.c.	0.02 gm.	6.0 c.c.	1.0 c.c.	5.0 c.c.	2.5 c.c.
20 min.	3.0 c.c.	2.0 c.c.	1.0 c.c.	0.01 gm.	4.0 c.c.	1.0 c.c.	3.0 c.c.	1.5 c.c.
40 min.	2.0 c.c.	1.2 c.c.	0.8 c.c.	0.008 gm.	2.5 c.c.	0.5 c.c.	2.0 c.c.	1.0 c.c.
1 hr.	1.0 c.c.	0.6 c.c.	0.4 c.c.	0.004 gm.	2.5 c.c.	1.3 c.c.	1.2 c.c.	0.6 c.c.
2 hrs.	0.5 c.c.	0.4 c.c.	0.1 c.c.	0.001 gm.	2.0 c.c.	1.3 c.c.	0.7 c.c.	0.35 c.c.
3 hrs.	0.5 c.c.	0.5 c.c.	0	Trace(?)	1.5 c.c.	0.9 c.c.	0.6 c.c.	0.3 c.c.
4 hrs.	0.5 c.c.	0.5 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.
Running water	1.0 c.c.	1.0 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.

[16] At the time indicated, 5 c.c. of the contents of the collodion sac were removed and examined for hydrochloric acid and $NaHCO_3$.

inactivation may be found in the gradual changes taking place in the salt content of the serum within the sac during dialysis. As may be expected, sodium chlorid passes out of the celloidin sac with a greater velocity than the remaining salts, such as carbonates or phosphates, and this together with an increase in volume (by endosmosis of water) results in removing the restraining power of the chlorids upon the dissociation of the carbonates. These salts then inactivate the complement.

A study of the comparative velocity of osmosis of sodium chlorid and Na_2CO_3 through a celloidin membrane is given in table XVI. At the time indicated, five cubic centimeters of the contents of the collodion sac were removed and examined for sodium chlorid and sodium bicarbonate.

SUMMARY.

It is generally accepted that complement may be split into a mid-piece and an end-piece. The mid-piece is thought to be in the globulin fraction, and the end-piece in the albumin fraction. The restoration of complement activity by putting together the albumin and globulin fractions does not prove, however, that each fraction contained a part of the complement, for the albumin fraction can be reactivated in the absence of the globulin fraction.

Complement-splitting as brought about by hydrochloric acid, carbon dioxid, and dialysis, is really an inactivation of the whole complement by certain acids or alkalis, either added in the free state to the serum, or liberated as a result of the dissociation of certain electrolytes.

That the whole complement, and not a part only, is present in the albumin fraction of the serum can be demonstrated by the removal of the inhibitory action of the acid or alkali. This can be effected by the addition, not only of alkali or acid, but also of any amphoteric substance. When hydrochloric acid, carbon dioxid, or dialysis are employed to produce the phenomenon known as complement-splitting, the complement is merely inactivated, not split.

BIBLIOGRAPHY.

Amako, T., *Ztschr. f. Immunitätsforsch.*, 1910, viii, 168.
Bernhardt, *Wien. klin. Wchnschr.*, 1911, xxiv, 606.

Bordet, J., *Ann. de l'Inst. Pasteur,* 1896, x, 193; 1898, xii, 688.
Brand, E., *Berl. klin. Wchnschr.,* 1907, xliv, 1075.
Buchner, H., *Arch. f. Hyg.,* 1893, xvii, 138.
Buchner, H., and Orthenberger, M., *Arch. f. Hyg.,* 1890, x, 149.
Ehrlich, P., and Morgenroth, J., *Berl. klin. Wchnschr.,* 1899, xxxvi, 481.
Ferrata, A., *Berl. klin. Wchnschr.,* 1907, xliv, 366.
Fränkel, E., *Ztschr. f. Immunitätsforsch.,* 1911, viii, 781; 1911, x, 388.
Hecker, R., *Arb. a. d. k. Inst. f. exper. Therap.,* 1907, No. 3, 39.
Guggenheimer, H., *Ztschr. f. Immunitätsforsch.,* 1911, viii, 295.
Kiss, J., *Ztschr. f. Immunitätsforsch.,* 1909–10, iv, 703; 1909, iii, 558.
Landsteiner, *Centralbl. f. Bakteriol., Ref.,* 1910, xlvii, Suppl., 88.
von Liebermann, L., *Arch. f. Hyg.,* 1907, lxii, 306; *Biochem. Ztschr.,* 1907, iv, 25.
Liefmann, H., *Centralbl. f. Bakteriol., Ref.,* 1910, xlvii, Suppl., 87; *München. med. Wchnschr.,* 1909, lvi, 2097.
Liefmann, H., and Cohn, M., *Ztschr. f. Immunitätsforsch.,* 1910, vi, 562; 1910, vii, 669; 1910, viii, 58.
Markl, *Ztschr. f. Hyg.,* 1902, xxxix, 86.
Marks, H. K., *Ztschr. f. Immunitätsforsch.,* 1910–11, viii, 508; 1911, xi, 18; *Jour. Exper. Med.,* 1911, xiii, 590.
Meyer, K., *Arch. f. Hyg.,* 1908, lxvii, 114.
Michaelis, L., and Skwirsky, P., *Ztschr. f. Immunitätsforsch.,* 1909–10, iv, 357, 629; *Berl. klin. Wchnschr.,* 1910, xlvii, 139.
Mutermilch, S., *Compt. rend. Soc. de biol.,* 1911, lxx, 577.
Noguchi, H., *Proc. Soc. Exper. Biol. and Med.,* 1907, iv, 45, 157; *Biochem. Ztschr.,* 1907, vi, 327.
Noguchi, H., and Bronfenbrenner, J., *Jour. Exper. Med.,* 1911, xiii, 43.
Rusznyák, S., *Ztschr. f. Immunitätsforsch.,* 1910–11, viii, 421.
Sachs, H., *Centralbl. f. Bakteriol., Ref.,* 1908, xlii, Suppl., 174.
Sachs, H., and Altmann, K., Kraus and Levaditi's Handbuch der Technik und Methodik der Immunitätsforschung, Jena, 1909, ii, 965; *Berl. klin. Wchnschr.,* 1908, xlv, 699.
Sachs, H., and Bolkowska, G., *Ztschr. f. Immunitätsforsch.,* 1910, vii, 778.
Sachs, H., and Teruuchi, Y., *Berl. klin. Wchnschr.,* 1907, xliv, 467, 520, 602.
Skwirsky, P., *Ztschr. f. Immunitätsforsch.,* 1910, v, 538.
Tsurusaki, H., *Biochem. Ztschr.,* 1908, x, 345.

A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING.

Second Paper: Splitting of the Complement without a Visible Alteration of the Proteid Constituents.*

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In the preceding communication we have dealt with various phenomena of complement-splitting in which the globulin fraction of the serum was precipitated and subsequently separated from the albumin fraction. In all these processes, according to current conceptions, the globulin fractions contained the mid-piece of complement, and the albumin fractions, the end-piece. Yet, as has already been pointed out, the so-called end-piece of the albumin portion depends in no way upon the introduction of the mid-piece in order to regain its original complement property, but can be reactivated by any reagent carrying the ions opposite in sign to those keeping the active atom-complex of complement temporarily inactive. The precipitation of the globulin fraction, therefore, was not essential in splitting the complement, but was merely an associated phenomenon. The correctness of the above deduction will be established by the experiments recorded in the following pages.

The so-called splitting of complement may be effected by certain procedures without causing the separation of the globulin fraction of complement. In one of these procedures, distilled water in certain proportions is used, and in another serum is treated with phosphates. The inactivation of complement by distilled water is doubtless due to the action upon the complement of dissociated ions of electrolytes contained in the serum, and the inactivation by a mix-

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ture of acid and alkaline phosphates (the acid being in excess) seems similar to the action of any other acid, but the presence of an alkaline phosphate modifies the reaction of the medium in such a manner that the globulin is kept in solution.

Although the phenomena of splitting complement as brought about by these chemical or physical interferences appear to be similar in mechanism to those effected by complement fixation, the complex biological reactions of the latter are difficult to analyze, and will be left for future study.

Under separate headings, we shall give the results of the other ways of splitting complement.

COMPLEMENT-SPLITTING WITH WATER.

Complement, as long as it is handled with an isotonic solution of sodium chlorid, retains its activity on dilution. On the other hand, complement rapidly loses its activity if it is diluted to a certain extent with an isotonic sugar (saccharose) solution, as was first pointed out by Sachs and Teruuchi. Thus these authors found that complement becomes completely inactive within one hour when diluted ten times with an isotonic sugar solution. The inactivation was considered by them to be due to a ferment which in a sodium chlorid-free medium manifests its activity upon complement.[1]

The above phenomenon appears, however, to be caused by some other factors than a ferment. In the case of inactivation by means of distilled water we must consider first what occurs if a solution containing two different salts of varying dissociabilities are diluted ten or more times with water. In the original concentration, the stronger electrolyte hinders the dissociation of the weaker, but when sufficiently diluted the dominating salt permits the weaker electrolyte to dissociate more fully. Sodium chlorid is a much stronger electrolyte than $NaHCO_3$ or Na_2CO_3 and it doubtless interferes with the free dissociation of the latter salts in the concentration present in serum; but when diluted ten times, its concentration no longer hinders the free dissociation of the carbonates. Thus, it becomes evident that the complement which may remain unaffected

[1] Bronfenbrenner, J., and Noguchi, H., *Jour. Exper. Med.*, 1912, xv, 598 (tables XIV and XV, A).

by the carbonates in the presence of sodium chlorid in the original concentration will be exposed to the action of the latter as soon as the sodium chlorid concentration is much less, and this is exactly what happens when serum is diluted with water. This, of course, will not occur as long as a sufficient amount of the chlorid is contained in the diluent (table I). That the change in the tonicity of the mixture (serum and diluent) has nothing to do with the loss of complement activity is shown by the fact that the use of an isotonic solution of a non-electrolyte, such as cane sugar, does not prevent the inactivation of complement.

We shall next see if the inactivation of complement by dilution with water or cane sugar solution is really an alkali inactivation. That this is the case is shown by the fact that an acid and also a few amphoteric substances may reactivate the inactive complement.[2]

TABLE I.

The Inactivation of Complement in a Salt-Free Isotonic Medium.

		Complement diluted to 40 % in 0.9 % NaCl and incubated at 37° C. for 1 hr.	Complement diluted to 40 % with 7.2 % sugar and incubated at 37° C. for 1 hr.
		0.2 c.c. of this dilution is used with varying amounts of 7.2 % sugar.	0.2 c.c. of this dilution is used with varying amounts of 0.9 % NaCl.
		Total volume made equal to 1 c.c. with 0.9 % NaCl.	Total volume made equal to 1 c.c. with 7.2 % sugar.
Varying amounts of sugar or NaCl added.	0.1	C.H.	No H.
	0.15	C.H.	Sl.H.
	0.2	C.H.	C.H.
	0.3	C.H.	C.H.
	0.4	C.H.	C.H.
	0.5	C.H.	C.H.
	0.7	C.H.	C.H.
	1 c.c.	C.H.	C.H.

COMPLEMENT-SPLITTING BY MEANS OF PHOSPHATES.

Michaelis and Skwirsky described a method by which complement can be split into end- and mid-pieces. In this method one cubic centimeter of complement (1:10) is mixed with two cubic centimeters of mixture A-B, which consists of sixteen parts of M/7 NaH_2PO_4 (solution A) and one part of M/7 Na_2HPO_4 (solution B), and to this are added one cubic centimeter of amboceptor (fifty

[2] Bronfenbrenner, J., and Noguchi, H., *loc. cit.*, tables XIV and XV.

TABLE II.

The Effect of Hydrochloric Acid upon the Inactivation of Complement by Distilled Water.

		0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.04 c.c.	0.03 c.c.	0.02 c.c.
Native complement + 0.9% NaCl added after 15 min.	1.0 c.c. 9.0 c.c.								C.H.	F.C.H.	Sl.H.	No H.
Complement + distilled water + 10% NaCl added after 15 min.	1.0 c.c. 8.2 c.c. 0.8 c.c.						C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
Complement + distilled water + 10% NaCl added after 30 min.	1.0 c.c. 8.2 c.c. 0.8 c.c.	No H.										
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 15 min.	1.0 c.c. 8.2 c.c. 0.8 c.c.		C.H.	F.C.H.	Sl.H.	Sl.H.	No H.					
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 30 min.	1.0 c.c. 8.2 c.c. 0.8 c.c.		C.H.	F.C.H.	Sl.H.	Sl.H.	No H.					
Complement HCl N/250 + NaOH N/25 in 10% NaCl added after 2 hrs.	1.0 c.c. 8.2 c.c. 0.8 c.c.		C.H.	F.C.H.	Sl.H.	Tr.H.	No H.					
Complement + 0.9% NaCl after 2 hrs. (control).	1.0 c.c. 9.0 c.c.								C.H.	Mch.H.		

units) and one cubic centimeter of 5 per cent. sheep corpuscles. After incubation for one hour at 37° C. the mixture is centrifugalized and the supernatant fluid is separated from the deposited corpuscles. To the supernatant fluid, two cubic centimeters of solution B are added and the whole is called the end-piece. The corpuscles are suspended in salt solution and are used as persensitized corpuscles (sensitized and carrying the mid-piece of complement). When the persensitized corpuscles are mixed with the end-piece they undergo hemolysis, while the sensitized corpuscles are not acted upon by the end-piece.

The above phenomenon is probably explained by the fact that complement is highly sensitive to the action of various acids, alkalis, and salts, and undergoes a reversible inactivation. It is not at all improbable, therefore, that the mixture of solutions A and B, which is strongly acid to phenolphthalein, renders the complement inactive, while the persensitization goes on without hinderance in the same medium. The acidity of the supernatant fluid is, in this instance, to a certain degree reduced by the later addition of solution B so that the inactivated complement, which is near the border of rever-

TABLE III.

Reaction and Anticomplementary Activity of Different Mixtures of Phosphates.

	Reaction of 1 c.c.		Anticomplementary activity upon 0.04 c.c. of complement + 2 units of amboceptor.	
	Indigo-alizarin.	Phenolphthalein.	Complete hemolytic dose.	Non-hemolytic dose.
Solution A. 27.4 gm. NaH_2PO_4 per 1,000 c.c.	Acid +0.4 c.c. NaOH N/100	Acid 24 c.c. NaOH N/100	0.07 c.c.	0.2 c.c.
Solution B. 51.4 gm. Na_2HPO_4 per 1,000 c.c.	Alkali 15 c.c. HCl N/100	Alkali 0.7 c.c. HCl N/100	0.5 c.c.	1 c.c.
Mixture A–B. 16 c.c. A+1 c.c. B.	Alkali 0.45 c.c. HCl N/100	Acid 22 c.c. NaOH N/100	0.1 c.c.	0.25 c.c.
Neutralized Mixture. 1 c.c. A–B+1 c.c. B.			0.5 c.c.	1 c.c.

sion, regains its activity whenever an adequate quantity of certain alkali (table IV) or amphoteric compounds is introduced (table V).

TABLE IV.

Reactivation of Complement Inactivated by Phosphates.

	NaOH N/1 in c.c.									
	0.1	0.07	0.05	0.03	0.02	0.01	0.005	0.001	0.0007	0.0005
Complement[3] 0.04 c.c. +27.4/1,000 NaH_2PO_4 0.2 c.c. in each tube + 3 units of amboceptor.	Alkali hemolysis.			C.H.	C.H.	C.H.	C.H.	No H.	No H.	No H.
	Zone neutral to phenolphthalein.							Zone neutral to alizarin-indigo-carmin.		

	HCl N/1 in c.c.										
	0.2	0.1	0.07	0.05	0.03	0.0275	0.025	0.02	0.01	0.007	0.005
Complement[3] 0.04 c.c. +51.4/1,000 Na_2HPO_4 1 c.c. in each tube + 3 units of amboceptor.	Acid hemolysis.										
			No H.	Sl.H.	C.H.	Sl.H.	No H.	No H.	No H.	No H.	No H.
	Zone neutral to phenolphthalein.								Zone neutral to alizarin-indigo-carmin.		

From the foregoing experiments it will be seen that the inactivated complement is to be found in the supernatant fluid in a reversible state. The reversion is accomplished by relieving the complement of the acid reaction by any suitable substance. The reason that the corpuscles digested in the Michaelis-Skwirsky mixture are capable of reversing the inactivated complement may be due to the absorption of a certain amount of serum constituents (probably the globulin fraction), also the absorption of the alkaline phosphate by certain constituents of the cells. It appears from the following experiments that the presence of amboceptor has a certain influence in enabling the corpuscles to absorb them, because without the amboceptor the persensitization is seen to be less complete (table VI).

That the end-piece of complement as obtained by the method of Michaelis and Skwirsky is in a general way similar to those obtained by other processes is well shown in the following experiments. It is most striking that alanin plays beautifully the rôle of the mid-piece with all end-pieces here studied (table VII, C).

[3] The complement was incubated with the phosphates at 37° C. for 30 minutes, then alkali and acid, respectively, were added and the whole was reincubated for 30 minutes.

TABLE V.

Reactivation of Complement Inactivated by Phosphates.

Complement 0.04 c.c. + Na_2HPO_4 (solution 51.4gm./1,000) 1 c.c. in each tube incubated for 30 minutes at 37° C. Then varying amounts of reactivating substances were added, the volume brought to 2 c.c. in each tube and incubated for 30 minutes at 37° C.; finally 10 units of amboceptor and 0.1 c.c. of a 10% suspension of corpuscles were added and again incubated for 30 minutes.											Na_2HPO_4. (No complement.)	
	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.	0.6 c.c.	0.7 c.c.	1 c.c.	Control.	0.5 c.c.	1 c.c.
Egg-white (1:5)					No H.	Sl.H.	F.C.H.	C.H.	C.H.			No H.
Serum 56° C. (sheep) ...			No H.	Sl.H.	Mch.H.	C.H.						No H.
Alanin 3% in NaCl (0.9%)	No H.	Sl.H.	F.C.H.	C.H.								No H.
HCl N/10 in 0.9% NaCl.	No H.	F.C.H.	C.H.	C.H.	Sl.H.	No H.					No H.	C.H.
o Noreactivating substances										No H.		

Same as above except that 0.2 c.c. NaH_2PO_4 (solution 27.4/1,000) was used instead of 1 c.c. of Na_2HPO_4.										NaH_2PO_4. (No complement.)	
	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	0.4 c.c.	0.5 c.c.	0.6 c.c.	0.7 c.c.	1 c.c.	Control.	0.3	0.5
Egg-white (1:5)				No H.	Sl. H.	F.C.H.	Sl.H.	No H.				
Serum 56° C. (sheep) ...	Tr.H.	Sl.H.	C.H.	C.H.	C.H.	F.C.H.	No H.					
Alanin 3% in 0.9% NaCl						No H.	No H.	Sl.H.	F.C.H.			
NaOH N/10 in 0.9% NaCl	F.C.H.	C.H.	C.H.	C.H.	F.C.H.	Sl.H.					No H.	C.H.
o Noreactivating substances										No H.		

TABLE VI.

Can Corpuscles be Persensitized without Amboceptor, or Complement, or Both?

Tubes.	1	2	3	4	5	6
	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Heated complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	0.9% NaCl 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. NaCl 0.9% 0.2 c.c. Corpuscles (5%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0.4 c.c. Amboceptor (20 units) 0.2 c.c. NaCl (0.9%) 0.2 c.c.	Complement (1:10) 0.2 c.c. Mixture A-B 0 Amboceptor 0 Corpuscles (5%) 0.2 c.c. NaCl (0.9%) 0.6 c.c.
Remarks		Heated complement	No complement	No amboceptor	No corpuscles	No mixture A-B No amboceptor
All mixtures incubated for 60 minutes at 37° C. and corpuscles separated by centrifugalization (to be used later).						
0.4 c.c. of solution B (Na_2HPO_4) added to all the supernatant fluids except the last (6).						0.4 c.c. NaCl added.
To the different amounts of the supernatant fluid of each tube the corpuscles of each of the other tubes were added and again incubated; results taken after 1 hour.						

(Continued on page 633.)

TABLE VI (*Continued*).

Corpuscles from tubes.	Supernatant fluid from tube 1.				Supernatant fluid from tube 2.			Supernatant fluid from tube 3.		
	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	1 c.c.	0.7 c.c.	0.5 c.c.	1 c.c.	0.7 c.c.	0.5 c.c.
1	C.H.	F.C.H.	Sl.H.	No H.	No H.			No H.		
2	C.H.	F.C.H.	Tr.H.	No H.	No H.			No H.		
3	C.H.	Mch.H.	No H.		No H.			No H.?		
4	F.C.H.	Sl.H.	No H.		No H.			No H.		
6	F.C.H.	Sl.H.	No H.		No H.			No H.		
Non-treated corpuscles (5%) 0.2 c.c.	F.C.H.	Sl.H.	No H.		No H.			No H.		

Corpuscles from tubes.	Supernatant fluid from tube 4.				Supernatant fluid from tube 5.				Supernatant fluid from tube 6.		
	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.
1	C.H.	F.C.H.	Tr.H.	No H.	C.H.	Sl.H.	No H.		C.H.	Mch.H.	No H.
2	C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.
3	C.H.	Mch.H.	Tr.H.	No H.	C.H.	Mch.H.	No H.		C.H.	Sl.H.	No H.
4	C.H.	Sl.H.	No H.		C.H.?	No H.	No H.		C.H.	Mch.H.	No H.
6	C.H.	Mch.H.	Tr.H.?	No H.	F.C.H.	Mch.H.	No H.		C.H.	Sl.H.	No H.
Non-treated corpuscles (5%) 0.2 c.c.	C.H.	Sl.H.	No H.		F.C.H.	Mch.H.	No H.		C.H.	Mch.H.	No H.
	Amboceptor (20 units) 0.2 c.c. added.								Amboceptor (20 units) 0.2 c.c. added.		

TABLE VII.

Activity of Different End-Pieces with Various Mid-Pieces and Alanin.

	0.5 c.c. of 5 % suspension of fresh corpuscles + 50 units of amboceptor.		Same amount of persensitized corpuscles.		Same amount of sensitized corpuscles.		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.5 c.c. of mid-piece (CO_2).		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.5 c.c. HCl mid-piece.		0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor + 0.3 c.c. of 3 % alanin.	
Amounts of end-piece used.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.	0.5 c.c.	0.3 c.c.
(A) End-piece (CO_2) made isotonic.	No H.	No H.	C.H.	C.H.	F.C.H.	C.H.	Mch.H.	No H.	C.H.	C.H.	C.H.	C.H.
(B) End-piece (HCl) made neutral and isotonic.	No H.	No H.	C.H.	C.H.	Sl.H.(?)	No H.	F.C.H.	Sl.H.	C.H.	C.H.	Sl.H.	No H.
Amounts of Skwirsky's[4] end-piece used.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.	2.5 c.c.	1.5 c.c.
(C) Skwirsky's end-piece neutralized by solution B.	No H.	No H.	C.H.	C.H.	Sl.H.(?)	No H.	C.H.	Sl.H.	C.H.	F.C.H.	C.H.	Sl.H.

[4] Skwirsky's end-piece being more diluted, it was necessary to increase the quantity accordingly.

COMPLEMENT-SPLITTING IN COMPLEMENT DEVIATION PHENOMENA.

Much more complicated is the phenomenon first described by Skwirsky, who found that the supernatant fluid of the mixture containing a positive syphilitic serum, syphilitic antigen, complement, sheep's corpuscles, and antisheep amboceptor, has the power to cause hemolysis upon a fresh lot of sheep corpuscles when the latter are previously persensitized. By the term of persensitization is meant the digestion of washed sheep corpuscles in a mixture of sixteen

TABLE VIII.

Action of Mixture A-B upon the Substances Concerned in the Wassermann Reaction.

1	Syphilitic serum (heated at 56° C.) 0.1 c.c. Antigen (0.3 %) (lipoids) 0.5 c.c. Complement (1:10) 0.5 c.c. NaCl (0.9%) 0.4 c.c. 1.5 c.c.	Each of the tubes contained this mixture (1.5 c.c.) The tubes were incubated at 37° C. for 30 min. then varying amounts of A–B mixture were added and the volume was brought up to 2.5 c.c. with NaCl (0.9 %). After the second incubation for 30 min. at 37° C., 0.5 c.c. of 5 % sheep corpuscles and 50 units of amboceptor were added and the whole was again incubated for 30 min. at 37° C.								
	Varying amounts of the mixture A–B.	1 c.c.	0.7c.c.	0.5c.c.	0.3c.c.	0.2c.c.	0.1c.c.	0.07c.c.	0.05c.c.	0
	Result of the hemolytic test.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
2	In this series, corpuscles were digested with amboceptor and mixture A–B for 30 min. at 37° C., and then to each tube 1.5 c.c. of the above mixture of complement, antigen, syphilitic serum, and NaCl were added. The amounts of substances used were the same as above.									
	Varying amounts of mixture A–B + corpuscles 5 % 0.5 c.c. + amboceptor 50 units.	1 c.c.	0.7 c.c.	0.5 c.c.	0.3c.c.	0.2 c.c.	0.1 c.c.	0.07c.c.	0.05c.c.	0
	Results of the hemolytic test after additions of 1.5 c.c. of the above mixture (1).	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	No H.
3	Corpuscles (0.5 c.c. of 5 % suspension) were digested for 30 min. at 37° C. with complement (1:10) 0.5 c.c. + amboceptor (50 units) 0.5 c.c. + mixture A–B 1 c.c.; then the corpuscles were separated by centrifugalization and acted upon by varying amounts of mixture 1.									
	Amounts of mixture 1 added; total volume made equal to 3.5 c.c.	1 c.c.	0.7c.c.	0.5c.c.	0.3c.c.	0.2 c.c.	0.1 c.c.	0		
	Results of the hemolytic experiment.	C. H.	C. H.	C. H.	Sl. H.	No H. (?)	No H.	No H.		

parts of M/7 solution of NaH_2PO_4 and one part of M/7 solution of Na_2HPO_4, to which a definite amount of complement and a large quantity of antisheep amboceptor have also been added (table IX, 2). The corpuscles digested in this mixture are hemolyzable by the supernatant fluid separated from a positive fixation test. The ordinary corpuscles with or without amboceptor are not hemolyzed by the supernatant fluid. Thus, this phenomenon was explained by them as being due to the presence of the end-piece of the complement in the supernatant fluid which, by virtue of the absorption of the mid-piece of complement by the sensitized corpuscles in the digestion procedure, becomes active upon the latter.

This phenomenon was confirmed by various investigators and we have also obtained a similar result (table IX).

Curiously enough, however, this apparent splitting of complement during the fixation by the Wassermann or Bordet-Gengou reaction does not take place when the antihuman hemolytic system is used (table X).

It is also interesting to note that the supernatant fluid of the Wassermann reaction which is quite active for the persensitized sheep corpuscles is almost inactive upon fresh sheep corpuscles when varying amounts of the mixture of solution A-B and fifty units of amboceptor are added (table XI, B). Apparently the addition of the A-B mixture and amboceptor alone is not sufficient to make the Wassermann supernatant fluid active. On the other hand, when the mixture A-B is allowed to act previously upon the sheep corpuscles with fifty units of antisheep amboceptor, the Wassermann supernatant fluid shows a certain amount of hemolytic effect. Here it seems as if the A-B mixture had so altered the corpuscles that they became to a certain extent hemolyzable by the Wassermann supernatant fluid (table XI).

It was also found that the persensitization of sheep corpuscles takes place in a hypertonic sodium chlorid or sugar solution, as indicated in the following experiments (table XII). At the same time it is interesting to notice that the Wassermann supernatant fluid is inactive upon the corpuscles persensitized when the medium is hypertonic.

The heating of the Wassermann supernatant fluid to 56° C. for

TABLE IX.

Splitting of Complement in the Wassermann Reaction with the Antisheep Hemolytic System.

1	2	3
Designated as Wassermann supernatant fluid.	Designated as persensitized corpuscles.	Designated as sensitized corpuscles.
Syphilitic serum (56° C.) 0.1 c.c. Antigen (lipoids) (0.3 %) 0.5 c.c. Complement (1:15) 0.5 c.c. NaCl (0.9 %) 0.4 c.c.	Mixture A–B 1 c.c. Corpuscles (5 %) 0.5 c.c. Amboceptor (50 units) 0.5 c.c. Complement (1:10) 0.5 c.c.	Suspension of corpuscles (10 %) 2.5 c.c. Amboceptor (50 units) 2.5 c.c.
Incubated 30 min. at 37° C.	Incubated 60 min. at 37° C. Then corpuscles were separated by centrifugalization and resuspended in 0.5 c.c. of 0.9 % NaCl.	Incubated 60 min. at 37° C. Then corpuscles were separated by centrifugalization and resuspended in 0.5 c.c. of 0.9 % NaCl.

Wassermann supernatant fluid (0.5 c.c. of complement 1:15 diluted in 1.5 c.c. of fluid).

Amounts of Wassermann supernatant fluid used.	+ 0.5 c.c. of persensitized corpuscles.		+ 0.5 c.c. of sensitized corpuscles.		
			+ 0	+ Mid-piece[5] (CO_2) 1:1	+ Alanin 3 % 0.3 c.c.
1.0 c.c.	C.H.		No H.	No H.	No H.
0.7 c.c.	C.H.				
0.5 c.c.	Sl.H.				
0.3 c.c.	No H.				
0.2 c.c.					

Complement (1:15) 0.5 c.c. + NaCl (0.9 %) 1 c.c. (0.5 c.c. of complement 1:15 diluted in 1.5 c.c. of salt solution).

Amounts of dilution of complement used.	+0.5 c.c. of persensitized corpuscles.		+ 0.5 c.c. of sensitized corpuscles.		
			+ 0	+ Mid-piece[5] (CO_2) 1:1	+ Alanin 3 % 0.3 c.c.
1.0 c.c.	C.H.		C.H.		
0.7 c.c.	C.H.		C.H.		
0.5 c.c.	Sl.H.		C.H.		
0.3 c.c.	No H.		F.C.H.	C.H.	C.H.
0.2 c.c.			Sl.H.	Mch.H.	Sl.H.
0.15 c.c.			No H.	No H.	No H.

[5] The amount of mid-piece used was equal to the amount of complement present in the corresponding amount of dilution used; namely, 1 c.c. of complement was broken up by CO_2 and the globulin fraction, collected by centrifugalization, dissolved in 15 c.c. of NaCl and 0.3 c.c., 0.2 c.c., 0.15 c.c., 0.07 c.c., respectively, were taken for 1 c.c., 0.7 c.c., 0.5 c.c., 0.3 c.c., of the Wassermann supernatant fluid.

TABLE X.

The Presence of End-Piece in the Supernatant Fluid of the Wassermann and Bordet-Gengou Reactions Tested with the Antihuman Hemolytic System.[6]

Designated as Wassermann supernatant fluid (comparable to table IX, 1).			
Syphilitic serum (56° C.) 0.1 c.c. Antigen (0.3 %) 0.5 c.c. Complement (1:15) 0.5 c.c. NaCl (0.9 %) 0.4 c.c.			
Incubated 30 min. at 37° C.			
	Mid-piece (CO_2).		No mid-piece (control).
	0.04 c.c.	0.02 c.c.	
The above Wassermann[7] supernatant fluid 1.5 c.c.	No H.	No H.	No H.
End-piece (CO_2) 0.04 c.c.	C.H.	C.H.	No H.
0 (control).	No H.		
	+ 0.5 c.c. of persensitized corpuscles.		
Wassermann supernatant fluid 1.5 c.c.	No H.		

Designated as mixture II.					
Egg-white (1:10) 1 c.c. Anti-egg serum (1:10) 1.5 c.c. NaCl (0.9 %) 7.5 c.c.					
Incubated 60 min. at 37° C., then shaken up and varying amounts incubated again with 0.1 c.c. of 40 % complement.					
	Amount of mixture II added to 0.1 c.c. of 40 % complement.				
	0	0.2 c.c.	0.4 c.c.	0.7 c.c.	1 c.c.
0.5 c.c. of 5 % fresh corpuscles + 50 units of amboceptor	C.H.	C.H.	C.H.	Sl. H.	No H.
0.5 c.c. of persensitized corpuscles.	C.H.	F.C.H.	Sl. H.	No H.	

Designated as supernatant fluid III.		
Sheep serum (56° C.) 0.1 c.c. Antisheep serum (56° C.) 0.1 c.c. Complement (40 %) 0.1 c.c. NaCl (0.9 %) 0.7 c.c.		
Incubated 60 min. at 37° C., centrifugalized and supernatant fluid examined.		
	+ 0.2 c.c. mid-piece.	No mid-piece.
Supernatant fluid III 1 c.c.	No H.	No H.
End-piece (CO_2) 0.04 c.c.	C.H.	No H.
	+ 0.5 c.c. of persensitized corpuscles.	
Supernatant fluid 1 c.c.	No H.	
Native complement 0.04 c.c.	C.H.	
End-piece (CO_2) 0.04 c.c.	C.H.	

[6] The table is arranged to show the three different procedures used in each of these experiments, all of which show the same result, contrary to the experiments with antisheep hemolytic system.

[7] In this experiment the human hemolytic system was used and in all the tubes the total volume was brought to 2 c.c. and when necessary 50 units of amboceptor and 0.5 c.c. of a 5 % suspension of human corpuscles were added.

TABLE XI.

The Effect of Mixture A-B upon Sheep Corpuscles and Wassermann Supernatant Fluid, Respectively.

	Amounts of solution A-B added.							0.5 c.c. of persensitized corpuscles.	0.5 c.c. of sensitized corpuscles.
								Controls.	Controls.
A. Wassermann[8] supernatant fluid 1.5 c.c. added to all. After additions of A–B, incubated at 37° C. for 30 min. 0.5 c.c. of 5% sheep corpuscles and 50 units amboceptor. Incubated for 60 min., and results taken.	1 c.c.	0.7 c.c.	0.5 c.c.	0.1 c.c.	0.5 c.c.	0.03 c.c.	0.01 c.c.	0	0
	No H.	No H.	No H.	No H.	No H.	No H.	No H.	C.H.	No H.
	Amounts of solution A-B added.							Controls	Controls
B. Corpuscles (5%) 0.5 c.c. Amboceptor (50 units) 0.5 c.c. Varying amounts of solution A-B. Incubated at 37° C., for 30 min., then[8] 1.5 c.c. Wassermann supernatant fluid added to each tube and after incubation for 60 min., results taken.	1 c.c.	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.	0.03 c.c.	0	0
	No H.(?)	Sl.H.(?)	Sl.H.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	No H.	C.H.	No H.

[8] See table IX, mixture I.

TABLE XII.

Does Persensitization Occur in a Hypertonic Medium?

Complement (1:15) 0.5 c.c. Amboceptor 50 units 0.5 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles 5% 0.5 c.c. 0.9 % NaCl solution 0.5 c.c.				Complement (1:15) 0.5 c.c. Amboceptor 50 units 0.5 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles 5% 0.5 c.c. 10% NaCl solution 0.5 c.c.		Complement (1:15) 0.5 c.c. Amboceptor (50 units) 0.5 c.c. Mixture A-B 1.0 c.c. Sheep corpuscles (5%) 0.5 c.c. 10 times isotonic (72%) sugar solution 0.5 c.c.		
After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.				After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.		After 30 min. incubation at 37° C., corpuscles separated and used for the following experiment.		
1.5 c.c. Wassermann supernatant fluid			1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of Wassermann supernatant fluid +0.5 c.c. of 0.9% NaCl	1.5 c.c. of 0.9% NaCl +0.5 c.c. of 0.9% NaCl	1.5 c.c. of Wassermann supernatant fluid +0.5 c.c. of 0.9% NaCl	
+0	+0.5 c.c. of 10% NaCl	+0.5 c.c. of 72% Sugar						
C.H.	No H.	F.C.H.	No H.	No H.	C.H.	No H.	C.H.	

TABLE XIII.

The Activation of Different End-Pieces by Different Mid-Pieces and Alanin in the Presence of Sheep or Human Corpuscles.

		End-piece (CO_2) isotonic, 0.4 c.c.		End-piece (HCl) neutralized and isotonic, 0.4 c.c.		Wassermann supernatant fluid, 1.5 c.c.		End-piece (Skwirsky)[9] neutralized by B, 3.5 c.c.		0.9% NaCl, 3.5 c.c.	
		Corpuscles.		Corpuscles.		Corpuscles.		Corpuscles.		Corpuscles.	
		Human.	Sheep.	Human.	Sheep.	Human.	Sheep.	Human.	Sheep.	Human.	Sheep.
Mid-piece (CO_2) 0.2 c.c.	50 units of amboceptor and 0.5 c.c. of 5% puscles	Sl.H.	Mch.H.	Sl.H.	C.H.	No H.	No H.	No H.	F.C.H.	No H.	No H.
Mid-piece (HCl) 0.2 c.c.		Mch.H.	F.C.H.	Mch.H.	C.H.	No H.	No H.	No H.	F.C.H.	No H.	No H.
Alanin (3%) 0.3 c.c.		Mch.H.	Mch.H.	C.H.	C.H.	No H.	No H.	No H.	No H.	No H.	No H.
Mid-piece of heated complement (CO_2) 0.2 c.c.		Mch.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	Sl.H.	No H.	No H.
0.5 c.c. of persensitized corpuscles.		Sl.H.	C.H.	Sl.H.	C.H.	No H.	F.C.H.	F.C.H.	C.H.	No H.	No H.

[9] Skwirsky's end-piece is the supernatant fluid (described in table IX, 2) resulting after persensitized corpuscles are removed from the mixture of A-B + complement + amboceptor + corpuscles. This supernatant fluid (2.5 c.c.) is to be neutralized by 1 c.c. of solution B and the result is designated Skwirsky's end-piece.

thirty minutes destroys its activity upon persensitized sheep corpuscles. The supernatant fluid from the positive Wassermann serum in which an inactivated complement, instead of fresh complement, had been used, is totally inactive upon the persensitized corpuscles, while the persensitization of the corpuscles can be accomplished by means of an inactivated as well as an active complement (table VI, 2).

From the fact that the sheep corpuscles separated from a positive Wassermann or Bordet-Gengou fixation test remain sensitized, but not persensitized, it is evident that the mid-piece in this instance interacts with the mixture of syphilitic serum and lipoids or antibody and antigen, thus becoming incapable of persensitizing the sensitized corpuscles. Just what kind of interaction has taken place is at present undetermined, but one point is certain and that is that the mid-piece is completely used up.

In this respect we may cite here an interesting phenomenon already observed by Noguchi and Bronfenbrenner (1910): namely, the interference exerted by certain amphoteric substances (egg-white, inactivated sera, etc.) upon the fixation phenomenon. Apparently the fixing molecules of the antibody-antigen combination are saturated by these substances which in this respect resemble the mid-piece.

GENERAL CONSIDERATION.

Thus far, most investigators have made but little distinction between the splitting phenomenon obtained by chemical interference and that which takes place in the biological phenomenon known as complement fixation. In this study we have shown that these two sets of phenomena have certain fundamental differences and that the so-called complement-splitting by physical conditions leading to chemical interaction, or directly by chemical means, is not a real splitting of the complement, but an inactivation of the active principle of complement through an alteration in the reaction of the medium caused by an excess of either anions or kations. The modification of the reaction of the medium may cause a more or less definite combination of the complement with the free ions, but the latter can readily be removed by an appropriate number of opposite

ions, and render the complement active once more. The fluids that have hitherto been regarded as containing the end-piece of complement, as a matter of fact, contain the whole complement temporarily deprived of its activity by certain ions derived either from the salt constituents of the serum itself under a modified physical condition (dialysis against water or dilution with water) or introduced in the form of dissociable electrolytes.

On the other hand, the splitting of complement in the fixation reaction seems far more complicated than that caused by the physical or chemical procedures. The supernatant fluid from the fixation test differs from all the other end-pieces prepared by chemical methods in being active upon persensitized sheep corpuscles only (not upon human corpuscles). The addition of various mid-pieces obtained by different methods to sensitized sheep corpuscles does not render the Wassermann supernatant fluid active (table XIII). It is quite remarkable that the persensitized sheep corpuscles are, on the other hand, easily attacked, not only by the supernatant fluids of fixation tests, but also equally well by the other end-pieces. It is not at all improbable that in the fixation reaction, where so many factors come into play, there is a most complicated physical as well as chemical interaction leading to such an entangled mixture of factors that a substance carrying one set of ions alone cannot reverse the activity of complement, and hence the reversion takes place only when certain electrolytes with both ions are employed. At all events there seems to be no doubt that the inactivation of complement is far more complicated in the Wassermann reaction or the Bordet-Gengou phenomenon than in the inactivation by physical or chemical means. Nevertheless, no one has as yet proved conclusively that the supernatant fluid of a fixation test necessarily contains the end-piece of complement.

A QUANTITATIVE STUDY OF THE EFFECTS OF ADRENALIN ON THE PUPILS OF RABBITS AFTER REMOVAL OF A SUPERIOR CERVICAL GANGLION.*

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Among the most characteristic effects of adrenin,[1] or epinephrin,[1] upon the animal body is its dilating action upon the pupil of mammals. It was first discovered by Lewandowsky[2] and confirmed by Boruttau[3] and Langley.[4] In their experiments, however, the dilatation produced was very transient, reaching its maximum within ten to thirty seconds after the intravenous injection and soon disappearing completely, the entire effect lasting two to four minutes at most. Subcutaneous injections or instillations into the conjunctival sac produced no effect, and the assumption was made that an early oxidation of the suprarenal extract within the tissues prevented its entrance into the blood-stream in the active form.

It was discovered later, however, by Meltzer and C. M. Auer[5] that the removal of a superior cervical ganglion favors greatly the dilating action of adrenin upon the pupil of the corresponding side, so that not only an intravenous, but even a subcutaneous injection, or an instillation in the corresponding conjunctival sac, caused a prompt dilatation of the corresponding pupil. Furthermore, with a sufficiently large dose of the adrenin, the dilatation of the pupil frequently lasted several hours. This is the more striking since it is in contrast to the fleeting effect which the administration of the

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[1] Designations for the active principle of adrenal extracts, in contradistinction to the terms employed for definite commercial preparations.

[2] Lewandowsky, *Arch. f. Physiol.*, 1899, 360.

[3] Boruttau, *Arch. f. d. ges. Physiol.*, 1899, lxxviii, 112.

[4] Langley, *Jour. Physiol.*, 1901–2, xxvii, 237.

[5] Meltzer and (C. M.) Auer, *Am. Jour. Physiol.*, 1904, xi, 28.

extract produced upon the pupil on the side with the intact ganglion. To obtain such striking effects the administrations of the adrenin had to be made some time after the removal of the ganglion, the necessary interval for rabbits being about twenty-four hours, and for cats about forty-eight hours.

The doses of adrenin employed in the experiments of Meltzer and Auer were calculated to bring out a maximum effect and therefore were quite large in most cases. In recent experiments upon the relations of the splanchnic nerves to the secretion of the adrenals[6] it became necessary to have some knowledge of the degree and duration of the effect which small doses of adrenin may exert upon the pupils. I have therefore carried out a series of experiments in which the dilating effect of adrenin upon the pupil was studied essentially from a quantitative point of view. This was done by the use of graded doses, and the attempt was made to find the smallest dose to which the deganglionized pupil will respond with a recognizable reaction, as well as to establish, if possible, the amount and duration of the dilatation produced by various larger doses. In addition, a few experiments were made to establish more exactly, how soon after the removal of the ganglion the increased sensitiveness of the pupil develops.

METHODS.

The experiments were made on rabbits. White ones were used exclusively, because of the ease with which the border of their pupils can be seen. From such rabbits, one superior cervical sympathetic ganglion was removed under morphin. The right and left ganglia were removed in about an equal number of animals. In the majority of the experiments the injections were made only after twenty-four hours, but in a few instances they were given during the first twenty-four hours in order to see how soon the increased sensitiveness appears. Only intravenous injections were used. In making the injections of adrenalin into a marginal ear vein the animals were held firmly in the hands of an assistant or were gently stretched out on a Cannon board. No anesthetic was required.

[6] Joseph and Meltzer, *Proc. Am. Physiol. Soc., Am. Jour. Physiol.*, 1912, xxix, p. xxxiv.

The solution used was the 1:1,000 adrenalin chlorid solution (Parke, Davis and Company). In nearly all cases it was used in this strength. In a few instances in which very small doses were given it was necessary, for accuracy, to dilute this solution to one half. The doses of adrenalin used, stated in terms of cubic centimeters of the 1:1,000 solution per kilo of body weight, were: 1/50, 1/30, 1/20, 1/10, and 2/10 of a cubic centimeter. A tuberculin (Record) syringe, graduated to fiftieths of a cubic centimeter, was used in making the injections.

After the injection the animal was released at once and the pupil readings were begun within a minute and repeated every few minutes while the dilatation lasted. The diameter of the pupil was estimated by comparing it with a pupillometer, consisting of a series of black discs of known diameter painted on a white background. The pupil was rarely round, and in the protocols, where two diameters are given for a single pupil, the first one is the horizontal as the animal stands erect, and the second, the vertical diameter. The latter is almost always the greater. The light conditions in individual experiments were as nearly constant as possible.

EXPERIMENTAL RESULTS.

Adrenalin, 1/50 of a Cubic Centimeter per Kilo of Body Weight.— In this series there were eight experiments (table I). In six of them the ganglion had been removed more than twenty-four hours previously. The dilatation produced was, with one exception, definite in all cases. It varied for the six animals between 0.5 of a millimeter (which is too small an amount to be considered a definite dilatation) and 3 millimeters, the average being 1.62 millimeters. The dilatation had reached its maximum in all cases within one minute after the injection. Within three to five minutes (average, four minutes) after the injection, a recovery of the pupil had begun. Recovery was complete in from five to twenty minutes (average, ten minutes) after the injection.

The quantity of adrenalin (0.02 of a milligram) employed in table I is almost the smallest to which the deganglionized pupil responds. A smaller amount could evidently not be depended upon to produce a dilatation. Control injections of a saline solution

TABLE I.

Adrenalin, 1/50 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.02 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
11	18 hours	0.75 mm.	———	5 minutes
12	18 hours	1.00 mm.	6 minutes	13 minutes
7	46 hours	0.75 mm.	4 minutes	10 minutes
8	46 hours	3.00 mm.	3 minutes	20 minutes
55	51 hours	2.00 mm.	5 minutes	6 minutes
56	51 hours	0.50 mm.	———	5 minutes
9	5 days	2.25 mm.	4 minutes	9 minutes
10	5 days	2.25 mm.	4 minutes	9 minutes
Averages for the last six experiments		1.62 mm.	4 minutes	10 minutes

The averages in this as well as in the four succeeding tables are derived only from the experiments performed twenty-four or more hours after the removal of the superior cervical ganglion.

were made, the same technique being used as for the adrenalin injections, but these caused no dilatation of the pupil.

Adrenalin, 1/30 of a Cubic Centimeter per Kilo of Body Weight.—In this series there were six animals (table II) in all of which the

TABLE II.

Adrenalin, 1/30 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.033 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
19	50 hours	1.50 mm.	7 minutes	7 minutes
20	52 hours	2.00 mm.	5 minutes	11 minutes
54	6 days	3.00 mm.	4 minutes	25 minutes
53	6 days	2.25 mm.	11 minutes	85 minutes
23	19 days	3.00 mm.	10 minutes	20 minutes
22	19 days	1.75 mm.	10 minutes	20 minutes
Averages for all six experiments		2.25 mm.	8 minutes	28 minutes.

ganglion had been removed over twenty-four hours before the injection. The maximum dilatation was observed in three of them within one minute after the injection, while in the three others the widest diameter was recorded only after five minutes. The amount

of dilatation varied between 1.5 and 3 millimeters, with an average of 2.25 millimeters. A beginning recovery from dilatation was noted between four and ten (average eight) minutes, which was complete in from seven to eighty-five (average, twenty-eight) minutes after the injection. There was one marked exception in the time between the injection and complete recovery. In five of the six experiments, recovery was complete in twenty-five minutes or less, whereas in one (rabbit 53) this period was eighty-five minutes in length. Were it not for this exception the average time between the injection and complete recovery would in this series be seventeen instead of twenty-eight minutes.

Adrenalin, 1/20 of a Cubic Centimeter per Kilo of Body Weight.—There were in this series fourteen experiments, thirteen of which were carried out more than twenty-four hours after gangliectomy (table III). In eleven of the thirteen experiments the dilatation had reached its maximum within one to two minutes after the injection, but in the remaining two it was maximal only after four minutes. It can be seen by a glance at table III that the amount of pupil dilatation produced with this dosage was much more constant than after 1/50 and 1/30 of a cubic centimeter of adrenalin,—this being especially

TABLE III.

Adrenalin, 1/20 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.05 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
3	19½ hours	1.25 mm.	6 minutes	13 minutes
6	43 hours	4.25 mm.	5 minutes	30 minutes
5	43 hours	2.50 mm.	8 minutes	39 minutes
1	48 hours	6.00 mm.	8 minutes	80 minutes
2	48 hours	4.00 mm.	11 minutes	75 minutes
33	3 days	3.50 mm.	9 minutes	30 minutes
34	3 days	3.50 mm.	———	50 minutes
15	4 days	3.50 mm.	4 minutes	22 minutes
14	4 days	2.50 mm.	4 minutes	14 minutes
38	5 days	2.75 mm.	6 minutes	20 minutes
13	6 days	3.75 mm.	7 minutes	11 minutes
41	9 days	3.50 mm.	6 minutes	44 minutes
49	14 days	3.25 mm.	4 minutes	26 minutes
28	19 days	3.75 mm.	5 minutes	48 minutes
Averages for the last thirteen experiments		3.60 mm.	6 minutes	37 minutes

evident in those animals in which the ganglion had been removed three days and longer. The maximum dilatation produced varied in the thirteen cases between 2.5 and 6 millimeters, with an average of 3.60 millimeters. Beginning recovery from dilatation was first noted in from four to eleven (average, six) minutes after the injection. Complete recovery had taken place in from eleven to eighty (average, thirty-seven) minutes after the injection. Here again, as with $\frac{1}{30}$ of a cubic centimeter per kilo, the greatest variations (rabbits 1 and 2) occur in rabbits in which the ganglion had been removed forty-eight hours or less.

Adrenalin, $\frac{1}{10}$ of a Cubic Centimeter per Kilo of Body Weight.—In this series there were twelve animals (table IV), in eight of which

TABLE IV.

Adrenalin, 1/10 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.01 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and the experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
42	4 hours	No dilatation—definite constriction.		
46	6 hours	No dilatation—definite constriction for 15 minutes.		
44	16 hours	½ millimeter dilatation followed in 3 minutes by constriction to a size less than normal.		
4	20 hours	3.50 millimeters	6 minutes	70 minutes
21	3 days	3.00 millimeters	6 minutes	28 minutes
30	4 days	4.25 millimeters	7 minutes	40 minutes
52	4 days	3.50 millimeters	5 minutes	38 minutes
32	5 days	4.25 millimeters	20 minutes	40 minutes
16	7 days	2.75 millimeters	7 minutes	53 minutes
17	8 days	4.50 millimeters	4 minutes	46 minutes
18	8 days	4.00 millimeters	19 minutes	65 minutes
48	15 days	4.50 millimeters	10 minutes	55 minutes
Averages for the last eight experiments		3.84 millimeters	10 minutes	45 minutes

the ganglion had been removed more than twenty-four hours before the injection of adrenalin. The time between the injection and the appearance of the maximum dilatation seemed in this series to be somewhat longer than with smaller doses. In three experiments this period was one minute; in one, two minutes; in three, four minutes; and in one, seven (average, three) minutes. In most, if not in all of the animals in which the maximum dilatation appeared late, there were rather marked symptoms produced by the adren-

alin. These symptoms were those of severe prostration, during which the animal's nose usually rested on the table. Occasionally, however, the animal might lie sprawled out for a few minutes, the respiration being deeper and more rapid than normally, the animal taking no interest in its surroundings, and making no attempt to move about. These symptoms were shown in varying degrees by most of the rabbits which received the larger doses; *i. e.*, $\frac{1}{10}$ and $\frac{2}{10}$ of a cubic centimeter per kilo. One rather striking effect of this kind will be mentioned in the account of the next series.

Here, as also with $\frac{1}{20}$ of a cubic centimeter per kilo, the amount of dilatation was fairly constant. It varied in the eight experiments between 2.75 and 4.5 millimeters, with an average of 3.84 millimeters. A beginning recovery was first noted between five and twenty (average, ten) minutes after the injection. Recovery from dilatation was complete in from twenty-eight to sixty-five (average, forty-five) minutes after the injection.

Adrenalin, $\frac{2}{10}$ of a Cubic Centimeter per Kilo of Body Weight.—In this series of eleven animals, a superior cervical ganglion had been removed twenty-four or more hours before the injection of adrenalin, the dose here employed, 0.2 of a cubic centimeter per kilo of body weight, being the largest used in any of the five series (table V). In seven of the eleven experiments the maximum dilatation

TABLE V.

Adrenalin, 2/10 of a Cubic Centimeter per Kilo of Body Weight of a 1:1,000 Solution (= 0.02 of a Milligram of Adrenalin per Kilo).

Number of experiment.	Time between removal of ganglion and experiment.	Amount of dilatation produced.	Time between injection and first sign of recovery of pupil.	Time between injection and complete recovery of pupil.
45	24 hours	5.50 millimeters	5 minutes	38 minutes
43	24 hours	4.00 millimeters	4 minutes	45 minutes
29	4 days	4.75 millimeters	8 minutes	95++ minutes
51	4 days	6.25 millimeters	27 minutes	51++ minutes
31	5 days	4.00 millimeters	30 minutes	110 minutes
39	6 days	4.25 millimeters	14 minutes	170 minutes
36	8 days	3.25 millimeters	13 minutes	76 minutes
37	8 days	4.25 millimeters	8 minutes	40 minutes
35	8 days	3.50 millimeters	41 minutes	145 minutes
40	9 days	5.75 millimeters	43 minutes	170++ minutes
47	14 days	2.50 millimeters	28 minutes	105++ minutes
Averages for all eleven of the experiments		4.36 millimeters	20 minutes	100+ minutes.

was recorded within one to two minutes after the injection. In the remaining four, this period was much longer, being respectively, ten, fourteen, seventeen, and twenty-two minutes in length, so that the average for the eleven animals, six and a half minutes, is considerably longer than in any of the other series with smaller doses of adrenalin.

I shall now describe in detail the conduct of the pupil and the symptoms following the injection of adrenalin into the animal in which the maximum dilatation appeared only after twenty-two minutes.

A white female rabbit, weighing 1,630 grams, received very slowly in an ear vein $\frac{2}{10}$ of a cubic centimeter per kilo of the adrenalin solution. In three minutes the pupil had increased 2.5 millimeters in diameter. It continued to increase for a short time until the total dilatation was 3 millimeters. Then serious symptoms developed. The animal which had up to this time appeared restless, sneezed repeatedly, brushed its nose with its paws, then jumped violently out of the box a number of times. The iris was very pale, and the heart was very rapid. Now and then the symptoms nearly disappeared and the animal sat quietly on its haunches. Respiration was deep and slightly more rapid than normally. During the attack the pupil became smaller and did not again dilate until the animal quieted down, when, twenty-two minutes after the injection, the point of maximum dilatation was reached.

The amount of dilatation produced in this series varied between 2.5 and 6.25 millimeters, with an average of 4.36 millimeters. In only three of the eleven experiments was the maximum amount of dilatation produced less than 4 millimeters, and in two of these it was 3.25 and 3.5 millimeters respectively. The first sign of recovery from dilatation was recorded between four and forty-three minutes after the injection, with an average of twenty minutes. In five of the eleven experiments the maximum dilatation persisted for from twen*y*-seven to forty-three minutes. This long continued maximum dilatation was more prominent with this dosage than with any other that was used. The return of the pupil to the normal diameter was also far more gradual than after smaller doses of adrenalin. The shortest interval between injection and com-

plete recovery of the pupil from dilatation was thirty-eight minutes, while in one case the recovery was not complete in one hundred and seventy minutes (about three hours). The average length of this interval is over one hundred minutes. It cannot be given accurately, for in several of the experiments the observations were discontinued for various reasons before complete recovery had taken place.

In considering the experiments in each of the foregoing series, I have thus far said nothing regarding those in which the injections were given during the first twenty-four hours after removal of the ganglion. By referring to tables I, III, and IV, and comparing in each table the response of the pupil to adrenalin during the first twenty-four hours after gangliectomy, with the response given at a later time to the same dose of adrenalin, the following points will be noted: Two injections (table IV), made four and six hours respectively after the removal of the ganglion, gave, not a dilatation, but a constriction of the pupil. In another experiment in the same table (rabbit 44), an injection sixteen hours after gangliectomy gave a dilatation of only 0.5 of a millimeter, which was followed in three minutes by a constriction that made the pupil smaller than normally. In still another animal (rabbit 4) an injection given after twenty hours gave a dilatation of 3.5 millimeters; *i. e.*, well up to the average for the whole series of injections given in this table.

In brief, then, these few injections indicate that for the first few hours after removal of the corresponding ganglion, the pupil is no more sensitive to the action of adrenalin than is the pupil of a normal animal; that within sixteen hours after gangliectomy, a faint dilatation may be obtained, while somewhere between the eighteenth and the twenty-fourth hour the sensitiveness rises rapidly to nearly or quite that which is seen at any later time.

The following protocols, one taken from each of the series with different doses of adrenalin, will serve to illustrate the course of the action of adrenalin. As already stated, where two diameters are given for a single pupil, the first is always the horizontal diameter as the animal stands erect, and the second the vertical diameter. In each protocol the pupil on the normal side serves as a control for the one whose ganglion has been removed.

PROTOCOL No. 1.

Rabbit 9. November 23, 1911. White Male; Weight, 1,790 Grams. Right Superior Cervical Ganglion Removed Five Days Ago.

Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
10:53	4.0	4.5	4.0	4.5
11:00	4.0	4.5	4.0	4.5
11:05	4.0	4.5	4.0	4.5
11:14	Received 1/50 c.c. per kilo of body weight of adrenalin into left ear vein.			
11:15	6.5		4.0	4.5
11:18	5.5		4.0	4.5
11:23	4.0	4.5	4.0	4.5
11:35	4.5		4.0	4.5
11:56	4.0	4.5	4.0	4.5
12:20	4.0	4.5	4.0	4.5

PROTOCOL No. 2.

Rabbit 23. December 9, 1911. White Male; Weight, 1,400 Grams. Left Ganglion Removed Nineteen Days Ago.

Time.	Size of right pupil (control) in mm.		Size of left pupil in mm.	
10:46	4.0	4.5	3.5	3.75
10:56	4.0	4.5	3.75	4.0
11:05	4.0	4.5	3.75	4.0
11:20	Received 1/30 c.c. per kilo of body weight of adrenalin into right ear vein.			
11:21	4.0	4.5	6.0	
11:24	4.0	4.5	6.5	7.0
11:30	3.75	4.0	5.5	6.0
11:40	4.0	4.5	3.75	4.0
12:00	4.0	4.5	4.5	
12:15	4.0	4.5	4.0	

PROTOCOL No. 3.

Rabbit 2. November 20, 1911. White Male; Weight, 1,315 Grams. Left Superior Cervical Ganglion Removed Forty-Eight Hours Ago.

Time.	Size of right pupil (control) in mm.		Size of left pupil in mm.	
11:10	5.5	6.0	6.0	6.5
11:17	5.5	6.0	6.0	6.5
11:21	5.5	6.0	6.0	6.5
11:35	Received 1/20 c.c. per kilo of body weight of adrenalin into right ear vein.			
11:35	5.5	6.0	10.0	
11:40	5.5	6.0	10.0	
11:46	6.0		9.0	
11:52	6.0	6.5	8.5	
12:00	6.0	6.5	8.0	
12:10	6.0	6.5	7.5	8.0
12:29	6.5		7.0	7.5
12:50	6.0	6.5	6.5	7.0
1:15	6.5		7.0	
1:28	6.0	6.5	6.5	7.0
1:45	6.0		6.5	7.0
2:08	6.0		6.5	7.0
4:15	6.5	7.0	6.5	7.0

PROTOCOL No. 4.

Rabbit 48. January 17, 1912. White Female; Weight, 1,600 Grams. Right Superior Cervical Ganglion Removed Fifteen Days Ago.

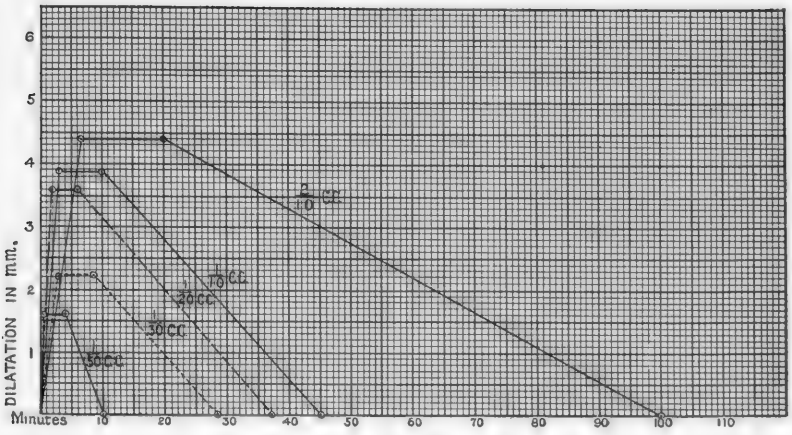
Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
1:18	4.75	5.75	5.25	6.25
1:28	5.25	5.75	4.75	5.5
1:33	4.5	5.0	4.75	5.25
1:35	Received 1/10 c.c. per kilo of body weight of adrenalin into left ear vein.			
1:36	7.5	8.5	4.75	5.75
1:39	9.0	9.25	4.5	5.0
1:45	8.0	8.25	4.75	5.25
1:55	7.25	8.0	4.75	5.5
2:07	7.0		4.75	5.0
2:30	5.75	6.0	4.75	5.5
3:13	5.25	5.75	4.75	5.5

PROTOCOL No. 5.

Rabbit 40. January 12, 1912. White Male; Weight, 1,675 Grams. Right Ganglion Removed Nine Days Ago.

Time.	Size of right pupil in mm.		Size of left (control) pupil in mm.	
10:44	3.75	4.25	3.75	4.10
10:52	3.75	4.0	4.5	5.0
10:56	Received 2/10 c.c. per kilo of adrenalin into left ear vein.			
10:57	9.5		3.75	4.5
11:02	8.0	9.0	2.5	3.0
11:07	8.5	9.0	3.5	3.75
11:16	8.0	8.5	3.75	4.0
11:26	9.0		4.0	4.5
11:39	8.0		3.5	4.0
11:51	7.0	7.5	4.0	4.5
12:06	7.0	7.5	4.0	4.5
12:21	7.0		4.5	5.25
12:29	6.5	7.25	4.5	5.25
1:15	5.75	6.25	4.25	5.0
2.04	5.0	5.5	4.0	4.75
3:14	4.75	5.75	4.0	4.5

In the following chart have been plotted the average figures obtained from each series of animals from which one ganglion had been removed twenty-four hours or more before the intravenous injection of adrenalin. The figures show the comparative effects of the different doses of adrenalin upon the pupil. The amount of dilatation is shown on the ordinates, taking the abscissa as the normal diameter before the injection of adrenalin, while along the abscissa is given the time in minutes after the injection.



TEXT-FIG. 1. For description see text.

SUMMARY AND CONCLUSIONS.

It has been shown by these experiments that after the removal of one superior cervical ganglion of a rabbit, $\frac{1}{50}$ of a cubic centimeter of a 1 : 1,000 adrenalin solution (=0.02 of a milligram of adrenalin) per kilo of body weight injected intravenously, is practically the minimum amount that will produce in nearly all cases a perceptible dilatation of the pupil. In one out of eight animals this dose failed to give a definite effect, while in two others the effect was slight.

Six experiments with injections of $\frac{1}{50}$ of a cubic centimeter of adrenalin per kilo of body weight gave the following averages: a maximum dilatation of 1.62 millimeters, which showed the first sign of decreasing in four minutes and had disappeared entirely in ten minutes after the injection.

Six experiments with $\frac{1}{30}$ of a cubic centimeter per kilo gave the following averages: a maximum dilatation of 2.25 millimeters, which began to disappear in eight minutes and had entirely disappeared in twenty-eight minutes.

For thirteen experiments with $\frac{1}{20}$ of a cubic centimeter of adrenalin per kilo these averages were: a maximum dilatation of 3.61

millimeters, a beginning recovery from dilatation in six minutes, with complete recovery of the pupil in thirty-seven minutes.

With $\frac{1}{10}$ of a cubic centimeter of adrenalin per kilo (eight experiments) the averages were: a maximum dilatation of 3.87 millimeters, a beginning recovery in ten minutes, with complete recovery of the pupil in forty-five minutes.

Finally, eleven experiments with $\frac{2}{10}$ of a cubic centimeter of adrenalin per kilo gave averages as follows: a maximum dilatation of 4.38 millimeters, a beginning recovery from dilatation in twenty minutes, with complete recovery only after one hundred minutes or more.

The time between the injection and the appearance of the maximum dilatation of the pupil varied somewhat according to the dose of adrenalin. In the series of experiments with $\frac{1}{50}$ of a cubic centimeter of adrenalin per kilo of body weight the average for this interval was one minute; in the series with $\frac{1}{30}$, $\frac{1}{20}$, and $\frac{1}{10}$ of a cubic centimeter the average length of this interval was about three minutes, while the experiments with $\frac{2}{10}$ of a cubic centimeter give us an average for this interval of six and a half minutes, with individual instances in which the maximum dilatation was seen only after fourteen, seventeen, and twenty-two minutes after the injection.

In those animals in which the maximum pupil dilatation was especially delayed, there were almost always more or less alarming symptoms of general prostration for a short time after the injection, and the maximum dilatation appeared as these symptoms gradually disappeared.

In other words, with an increase in the size of the dose of adrenalin, there was a gradual increase in the following: (1) the time between the injection and the appearance of the maximum dilatation; (2) the amount of dilatation produced; (3) the interval between the injection and the beginning of recovery from dilatation; and (4) in the total time between the injection and the return of the pupil to its normal size.

After removal of the ganglion a certain time must elapse before the increase in the sensitiveness develops. During the first ten to fifteen hours there is practically no increase in the sensitiveness. After eighteen hours a moderate effect can be obtained which rap-

idly increases so that by twenty to twenty-four hours after the removal of the ganglion any given dose of adrenalin produces practically as great a dilatation as it will give at any later time.

The experiments teach us that, on account of the individual variation in the degree of dilatation produced by a given dose of adrenin, we may not determine with exactness from any given degree of dilatation of the pupil the quantity of adrenin injected into the blood stream, and that we can not state with absolute exactness either the smallest dose that will constantly cause a dilatation, or mention the exact amount of adrenin which will in no case cause a dilatation of the pupil. However, the results permit the general statement that very small doses of adrenin on entering the circulation cause a fairly prolonged definite dilatation of a deganglionized pupil.

Since this is true, there are then at least two ways in which a rabbit from which one superior cervical ganglion has been previously removed may be used for determining qualitatively the amount of adrenin present. If, for example, we have a solution which may or may not contain adrenin, and upon injecting it obtain a dilatation of the deganglionized pupil only while the pupil on the normal side remains unchanged in size, we are justified in assuming that the solution contains adrenin. Again, if after stimulation of the peripheral end of a splanchnic nerve or other procedure upon the animal body under conditions that exclude the possibility of reflex effects, a dilatation of the deganglionized pupil results, while its normal mate remains unchanged, the assumption is warranted, though not absolutely proved, that some adrenin has been thrown into the circulation.

This test for the presence of adrenin has the advantage over other tests, that the pupil on the normal side will always act as a control.

INDEX TO VOLUME XV.

ADRENALIN, constricting influence of, upon the human coronary arteries, 404
Adrenalin, effects of, on the pupils of rabbits after removal of a superior cervical ganglion, 644
Adrenalin, existence of, in pathological sera, 547
Albuminolysins and their relation to precipitin reactions, 529
Anesthesia, chloroform, and pregnancy, 246
Antibodies, production of, by tissues living outside of the organism, 287
Aortic transplantations, ultimate results of, 389
Appendix, lesions produced in the, by *Oxyuris vermicularis* and *Trichocephalus trichiura*, 225
Arteries, human coronary, constricting influence of adrenalin upon, 404
Arteries in shock, 415
Augmented breathing, pressor influence of, 174
Auricular tachycardia, 49
AUSTRIAN, CHARLES R. The production of passive hypersensitiveness to tuberculin. A preliminary report, 149
Autograft, parathyroid, report of a dog maintained in good health by, 205
Autolysis and necrosis, correlation of the histological and chemical changes in the spleen during, 429

BACTERIAL endocarditis, subacute, glomerular lesions of, 330
BAEHR, GEORGE. Glomerular lesions of subacute bacterial endocarditis, 330
BAILEY, C. H. Effects on titrations of inequality of sensitization of corpuscles, 470
BARBOUR, HENRY G. The constricting influence of adrenalin upon the human coronary arteries, 404
BARTLETT, WILLARD. An experimental study of the arteries in shock, 415
Biochemical study of the phenomena known as complement-splitting, 598, 625
Blood pressure records, continuous, in man, 370
Blood pressure, respiratory waves of, 370
Breathing, augmented, pressor influence of, 174
BRONFENBRENNER, JACOB, and NOGUCHI, HIDEYO. A biochemical study of the phenomena known as complement-splitting. First paper: Splitting of the complement associated with globulin precipitation, 598
——. A biochemical study of the phenomena known as complement-splitting. Second paper: Splitting of the complement without a visible alteration of the proteid constituents, 625
BROWN, WADE H. Malarial pigment (hematin) as a factor in the production of the malarial paroxysm, 579
Bulkhead suture of the intestine, 216
BULKLEY, KENNETH. See CECIL and BULKLEY, 225

CALCIUM in intestinal infantilism, 450
Calorimeter experiments, and nitrogen and sulphur distribution in intestinal infantilism, 113
Cancers of the white rat, immunity in, 163
Carbohydrate and fat diets, effect of, on the excretion of creatin in cases of retarded development, 457
Carcinoma, presence of nuclease in, 477
CARREL, ALEXIS. Ultimate results of aortic transplantations, 389
——. Technique for cultivating a large quantity of tissue, 393
——. On the permanent life of tissues outside of the organism, 516
CARREL, ALEXIS, and INGEBRIGTSEN, RAGNVALD. The production of antibodies by tissues living outside of the organism, 287
CECIL, RUSSELL L., and BULKLEY,

KENNETH. On the lesions produced in the appendix by *Oxyuris vermicularis* and *Trichocephalus trichiura*, 225
Cells, foreign body giant, production of, *in vitro*, 510
Chemical and histological changes in the spleen during necrosis and autolysis, 429
Chemical studies on intestinal infantilism, 107, 113
Chloroform anesthesia and pregnancy, 246
Chloroform poisoning, insusceptibility of pups to, 259
Chronic valvular disease, effects of vagus stimulation on the hearts of children with, 14
Circle of Willis, function of, 348
COHN, ALFRED E. Auricular tachycardia with a consideration of certain differences between the two vagi, 49
Complement-splitting associated with globulin precipitation, 598
Complement-splitting, biochemical study of the phenomena known as, 598, 625
Complement-splitting without visible alteration of proteid constituents, 625
Constricting influence of adrenalin upon the human coronary arteries, 404
Coronary arteries, human, constricting influence of adrenalin on, 404
CORPER, HARRY J. Correlation of the histological and chemical changes in the spleen during necrosis and autolysis, 429
Corpuscles, effects on titrations of inequality of sensitization of, 470
COURET, MAURICE. See DUVAL and COURET, 292
Creatin, excretion of, in cases of retarded development, 457
Creatin, see also kreatin, 107
Cultivation, direct, of *Treponema pallidum* pathogenic for the monkey, 90
Cultivation of a large quantity of tissue, technique for, 393
Cultivation of *Spirochæta refringens*, 466
Cultural studies on mouth spirochætæ, 81
Culture media for growing tissues, influence of heat on different sera as, 397

DARLING, S. T. Experimental infection of the mule with *Trypanosoma hippicum* by means of *Musca domestica*, 365
——. The infection of mules by *Trypanosoma hippicum* through mucous membranes, 367
DIXON, ROBERT L. The effects of splenectomy upon the cell content of the thoracic duct lymph in the dog, and its relation to the action of pilocarpin, 63
DRAPER, GEORGE. See ROBINSON and DRAPER, 14
DUVAL, CHARLES W., and COURET, MAURICE. A further note upon the experimental production of leprosy in the monkey (*Macacus rhesus*) with a critical study of the culture employed, 292

EBERLY, K. C. See WIGGERS, EBERLY, and WENNER, 174
Electrocardiograph, studies with the, on the action of the vagus nerve on the human heart, 14
Embryo, developing, behavior of chicken sarcoma implanted in the, 119
Endocarditis, subacute bacterial, glomerular lesions of, 330
Endogenous metabolism in intestinal infantilism, 107, 113
Epinephrin, existence of, in pathological sera, 547
EPSTEIN, ALBERT A. Immunochemical studies with peptones. I. General studies with peptones and the immune hemolytic system, 485
ERLANGER, JOSEPH, and FESTERLING, E. G. Respiratory waves of blood pressure, with an investigation of a method for making continuous blood pressure records in man, 370
Excretion of creatin in cases of retarded development, 457
Exogenous metabolism in intestinal infantilism, 113
Experimental infection of the mule with *Trypanosoma hippicum*, 365, 367
Experimental leprosy, 292
Experimental pneumonia by intrabronchial insufflation, 133
Experimental studies of the arteries in shock, 415
Experimental studies on the administration of salvarsan by mouth to animals and man, 498

Experimental tuberculous meningitis, 1

FALES, HELEN L. See MCCRUDDEN and FALES, 113, 450
Fat and carbohydrate diets, effect of, on the excretion of creatin, in cases of retarded development, 457
FESTERLING, E. G. See ERLANGER and FESTERLING, 370
Fetal tissues, 246
Foreign body giant cells, production of, *in vitro*, 510

GANGLION, superior cervical, effects of adrenalin on the pupils of rabbits after removal of a, 644
Giant cells, foreign body, production of, *in vitro*, 510
Globulin precipitation, splitting of complement associated with, 598
Glomerular lesions of subacute bacterial endocarditis, 330
GOODMAN, EDWARD H. On the presence of nuclease in carcinoma, 477
GRAHAM, EVARTS A. The pathogenesis of the hemorrhagic diseases of the new-born, 307
Graves' disease, development of the operation for, 205

HALSTED, WILLIAM S. Report of a dog maintained in good health by a parathyroid autograft approximately one fourth of a millimeter in diameter. And comments on the development of the operation for Graves' disease as influenced by the results of experiments on animals, 205
——. A bulkhead suture of the intestine, 216
Heart, human, studies with the electrocardiograph of the action of the vagus nerve on, 14
Heat, influence of, on different sera as culture media for growing tissues, 397
Hematin as a factor in the production of the malarial paroxysm, 579
Hemolytic system, immune, and peptones, 485
Hemorrhagic diseases of the new-born, pathogenesis of, 307
Histological and chemical changes in the spleen during necrosis and autolysis, 429
Histological signs of resistance to a transmissible sarcoma of the fowl, 270
Hypersensitiveness, passive, to tuberculin, production of, 149

IMMUNE hemolytic system and peptones, 485
Immunity in cancers of the white rat, 163
Immunochemical studies with peptones, 485
Infantilism, intestinal, chemical studies on, 107, 113
Infection, experimental, of the mule with *Trypanosoma hippicum*, 365, 367
INGEBRIGTSEN, RAGNVALD. The influence of heat on different sera as culture media for growing tissues, 397
——. See CARREL and INGEBRIGTSEN, 287
Injections of leucocytes, effects of, on experimental tuberculous meningitis, 1
Insufflation, intrabronchial, experimental pneumonia by, 133
Intestinal infantilism, 107, 113, 450
Intestine, a bulkhead suture of the, 216
Intrabronchial insufflation, experimental pneumonia by, 133
In vitro production of foreign body giant cells, 510
Islands of Langerhans after ligation of the pancreatic ducts, 101

JOSEPH, DON R. A quantitative study of the effects of adrenalin on the pupils of rabits after removal of a superior cervical ganglion, 644

KIRKBRIDE, MARY B. The islands of Langerhans after ligation of the pancreatic ducts, 101
KOLMER, JOHN A., and SCHAMBERG, JAY FRANK. Experimental studies on the administration of salvarsan by mouth to animals and man, 498
KRAMER, S. P. On the function of the circle of Willis, 348
Kreatin in intestinal infantilism, 107
Kreatin, see also creatin, 457
Kreatinin in intestinal infantilism, 107

LAMAR, R. V., and MELTZER, S. J. Experimental pneumonia by intrabronchial insufflation, 133
LAMBERT, ROBERT A. The production of foreign body giant cells *in vitro*, 510

Langerhans, islands of, after ligation of the pancreatic ducts, 101
Leprosy, experimental, in the monkey, 292
Lesions, glomerular, of subacute bacterial endocarditis, 330
Lesions produced in the appendix by *Oxyuris vermicularis* and *Trichocephalus trichiura*, 225
Leucocytes, effects of subdural injections of, on experimental tuberculous meningitis, 1
LEVIN, ISAAC. Studies on immunity in cancers of the white rat. The "precancerous state" and the mechanism of the organ resistance to tumor growth, 163
Lymph of the thoracic duct in the dog, effects of splenectomy upon the cell content of, 63

McCRUDDEN, FRANCIS H. Chemical studies on intestinal infantilism. I. Endogenous metabolism: kreatinin, kreatin, uric acid, 107
——. The effect of fat and of carbohydrate diets on the excretion of creatin in cases of retarded development, 457
McCRUDDEN, FRANCIS H., and FALES, HELEN L. Chemical studies on intestinal infantilism. II. The relation of endogenous to exogenous metabolism: nitrogen and sulphur distribution and calorimeter experiments, 113
——. Complete balance studies of nitrogen, sulphur, phosphorus, calcium, and magnesium in intestinal infantilism, 450
Magnesium in intestinal infantilism, 450
Malarial pigment (hematin) as a factor in the production of the malarial paroxysm, 579
MANWARING, WILFRED H. The effects of subdural injections of leucocytes on the development and course of experimental tuberculous meningitis, 1
Maternal tissues, 246
Media for growing tissues, influence of heat on different sera as, 397
MELTZER, S. J. See LAMAR and MELTZER, 133
Membranes, mucous, the infection of mules by *Trypanosoma hippicum* through, 367
Meningitis, experimental tuberculous, effects of subdural injections of leucocytes on, 1
Metabolism, endogenous, in intestinal infantilism, 107, 113
Metabolism, exogenous, in intestinal infantilism, 113
Morphological and pathogenic variations in *Treponema pallidum*, 201
Mouth spirochætæ (*Treponema microdentium* and *macrodentium*), cultural studies on, 81
Mucous membranes, infection of mules by *Trypanosoma hippicum* through, 367
MURPHY, JAMES B., and ROUS, PEYTON. The behavior of chicken sarcoma implanted in the developing embryo, 119
MURPHY, JAMES B. See ROUS and MURPHY, 270
Musca domestica, experimental infection of the mule with *Trypanosoma hippicum* by means of, 365

NECROSIS and autolysis, correlation of the histological and chemical changes in the spleen during, 429
Nerve, vagus, action of, on the human heart, 14
New-born, pathogenesis of the hemorrhagic diseases of the, 307
Nitrogen and sulphur distribution and calorimeter experiments in intestinal infantilism, 113
Nitrogen in intestinal infantilism, 450
NOGUCHI, HIDEYO. Cultural studies on mouth spirochætæ (*Treponema microdentium* and *macrodentium*), 81
——. The direct cultivation of *Treponema pallidum* pathogenic for the monkey, 90
——. Morphological and pathogenic variations in *Treponema pallidum*, 201
——. Pure cultivation of *Spirochæta refringens*, 466
NOGUCHI, HIDEYO. See BRONFENBRENNER and NOGUCHI, 598, 625
Nuclease in carcinoma, 477

OXYURIS *vermicularis* and *Trichocephalus trichiura*, lesions produced in the appendix by, 225

PANCREATIC ducts, islands of Langerhans after ligation of, 101
Parathyroid autograft, report of a

dog maintained in good health by, 205
Paroxysm, malarial, malarial pigment (hematin) as a factor in the production of, 579
Passive hypersensitiveness to tuberculin, production of, 149
Pathogenesis of the hemorrhagic diseases of the new-born, 307
Pathogenic and morphological variations in *Treponema pallidum*, 201
Pathological sera, existence of adrenalin in, 547
Peptones, immunochemical studies with, 485
Phosphorus in intestinal infantilism, 450
Pigment, malarial, (hematin), as a factor in the production of malarial paroxysm, 579
Pilocarpin, effects of splenectomy upon the cell content of the thoracic duct lymph in the dog, and its relation to the action of, 63
Placental tissues, 246
Pneumonia, experimental, by intrabronchial insufflation, 133
Poisoning, chloroform, insusceptibility of pups to, 259
"Precancerous state" and the mechanism of the organ resistance to tumor growth, 163
Precipitation, globulin, splitting of complement associated with, 598
Precipitin reactions, their relation to albuminolysins, 529
Pregnancy and chloroform anesthesia, 246
Pressor influence of augmented breathing, 174
Proteid constituents, splitting of complement without visible alteration of, 625
Pupils of rabbits, effects of adrenalin on, after removal of a superior cervical ganglion, 644

REACTIONS, precipitin, their relation to albuminolysins, 529
Refringens, Spirochæta, cultivation of, 466
Respiratory waves of blood pressure, 370
Retarded development, effect of fat and carbohydrate diets on the excretion of creatin in cases of, 457
ROBINSON, G. CANBY, and DRAPER, GEORGE. Studies with the electrocardiograph on the action of the vagus nerve on the human heart. II. The effects of vagus stimulation on the hearts of children with chronic valvular disease, 14
ROUS, PEYTON, and MURPHY, JAMES B. The histological signs of resistance to a transmissible sarcoma of the fowl, 270
ROUS, PEYTON. See MURPHY and ROUS, 119

SALVARSAN, administration of, by mouth to animals and man, 498
Sarcoma of the chicken, implanted in the developing embryo, 119
Sarcoma, transmissible, of the fowl, 270
SCHAMBERG, JAY FRANK. See KOLMER and SCHAMBERG, 498
Sensitization of corpuscles, effects on titrations of inequality of, 470
Sera, different, as culture media for growing tissues, influence of heat on, 397
Sera, pathological, the alleged existence of adrenalin in, 547
Shock, arteries in, 415
Spirochæta refringens, pure cultivation of, 466
Spirochætæ of the mouth, cultural studies on, 81
Spleen during necrosis and autolysis, correlation of the histological and chemical changes in, 429
Splenectomy, effects of, upon the cell content of the thoracic duct lymph in the dog, 63
STEWART, G. N. The alleged existence of adrenalin (epinephrin) in pathological sera, 547
Subacute bacterial endocarditis, glomerular lesions of, 330
Subdural injections of leucocytes, effects of, on experimental tuberculous meningitis, 1
Sulphur and nitrogen distribution and calorimeter experiments in intestinal infantilism, 113
Sulphur in intestinal infantilism, 450
Suture, bulkhead, of the intestine, 216

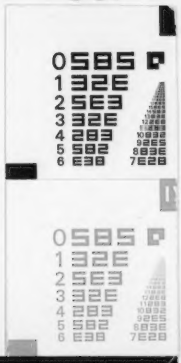
TACHYCARDIA, auricular, 49
Technique for cultivating a large quantity of tissue, 393
Thoracic duct lymph in the dog, effects of splenectomy upon the cell content of the, 63
Tissue, technique for cultivating a large quantity of, 393

Tissues, influence of heat on different sera as culture media for, 397
Tissues living outside of the organism, the production of antibodies by, 287
Tissues, maternal, placental, and fetal, 246
Tissues outside of the organism, permanent life of, 516
Titrations, effects on, of inequality of sensitization of corpuscles, 470
Transmissible sarcoma of the fowl, the histological signs of resistance to a, 270
Transplantations, aortic, ultimate results of, 389
Treponema microdentium and *macrodentium,* cultural studies on, 81
Treponema pallidum, morphological and pathogenic variations in, 201
Treponema pallidum pathogenic for the monkey, direct cultivation of, 90
Trichocephalus trichiura and *Oxyuris vermicularis,* lesions produced in the appendix by, 225
Trypanosoma hippicum, experimental infection of the mule with, by means of *Musca domestica,* 365
Trypanosoma hippicum, infection of mules by, through mucous membranes, 367
Tuberculin, production of passive hypersensitiveness to, 149
Tuberculous meningitis, experimental, effects of leucocytes on, 1
Tumor growth, "precancerous state" and the mechanism of the organ resistance to, 163

URIC acid in intestinal infantilism, 107

VAGI, certain differences between the two, 49
Vagus nerve, action of, on the human heart, 14
Vagus stimulation, effects of, on the hearts of children with chronic valvular disease, 14
Valvular disease, chronic, effects of vagus stimulation on the hearts of children with, 14
Variations, morphological and pathogenic, in *Treponema pallidum,* 201

WENNER, H. L., JR. See WIGGERS, EBERLY, and WENNER, 174
WHIPPLE, G. H. Pregnancy and chloroform anesthesia. A study of the maternal, placental, and fetal tissues, 246
——. Insusceptibility of pups to chloroform poisoning during the first three weeks of life, 259
WIGGERS, C. J., EBERLY, K. C., and WENNER, H. L., JR. The pressor influence of augmented breathing, 174
Willis, circle of, function of the, 348

ZINSSER, HANS. On albuminolysins and their relation to precipitin reactions, 529

C 37